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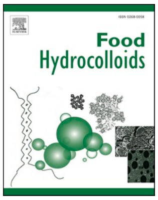

Granulation and encapsulation of *N*-Acetylcysteine (NAC) by internal phase separation

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ABSTRACT

The encapsulation of small hydrophilic molecules offers advantages for the delivery of hydrophilic food supplements and food ingredients. These advantages include: masking off-flavors, reducing astringency, and controlling release profiles. We used an internal phase separation (IPS) method for simultaneous granulation and encapsulation of a small hydrophilic food supplement in corn oil. To prove that IPS is a suitable method for the encapsulation of small hydrophilic molecules with controllable release times, we used the nutritional supplement *N*-acetylcysteine (NAC) as our model molecule. NAC has a variety of health and pharmaceutical benefits however it suffers from poor organoleptic properties such as a bitter aftertaste, astringency, and a strong sulfur smell. We used IPS to produce free-flowing granules containing 56–78% NAC with diameters ranging from 100 to 1000 μm. All the materials used in this process were either food safe or generally recognized as safe (GRAS). The granulated/encapsulated samples were characterized using FTIR, NMR, SEM, and LC-MS methods to confirm the presence of unreacted NAC in high loading concentrations. The kinetics of NAC release was then monitored using conductometry. The combination of hydrophilic carboxymethyl cellulose sodium salt (CMC) and hydroxypropyl methylcellulose (HPMC) gums with a hydrophobic coating such as ethyl cellulose (EC), provided an effective barrier to ensure a 5 min time window for NAC dispersion into water and drinks before the capsule starts to dissolve in water and loses more than 30 wt % of its total NAC loading.

1. Introduction

The encapsulation of small hydrophilic molecules is frequently used in the pharmaceutical and food industries. For example, encapsulation has been used to protect the drug and the digestive tract from unwanted side effects and to allow for controlled time release of drugs (Ye & Chi, 2018). Further, encapsulation technologies have also been used to stabilize food colorants and to protect or mask odors (Madene, Jacquot, Scher, & Desobry, 2006). There are many encapsulation methods reported in the literature, but for our purposes, we have investigated the use of internal phase separation (IPS) for the encapsulation of hydrophilic food ingredients (Trojer, Li, Abrahamsson, et al., 2013; Trojer, Li, Wallin, Holmberg, & Nydén, 2013). In IPS, an organic phase composed of two different solvents, one to dissolve the shell material and one to dissolve the core material, the encapsulant, is dispersed within the aqueous phase. The solvent for the shell material is chosen to be more volatile than the solvent for the encapsulant. This causes the dispersed organic phase to turn into two phases with one of the components migrating to the interface and forming the shell material, and the other component staying inside the particle and forming the core (Trojer, Li, Abrahamsson, et al., 2013). The selection of materials and solvents is critical to the success of the IPS process and most reports use non-food safe materials. To investigate the robustness of IPS and to show that it can be used with food-safe materials we used *N*-acetylcysteine (NAC) as a model compound. Small hydrophilic nutraceuticals are ideal candidates for encapsulation as they are often plagued by unfavorable organoleptic properties and benefit from controlled-release (Reque & Brandelli, 2021).

NAC is a hydrophilic nutraceutical that serves as an antioxidant, a precursor for glutathione, and a supplement form of cysteine. NAC has been used as a mucolytic agent and antidote for paracetamol overdose (Holdiness, 1991; Jaya, Augustine, & Menon, 1994; Mant, Tempowski,

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Volans, & Talbot, 1984) and alcohol intoxication (Jaya et al., 1994), and is sold as an over-the-counter nutritional supplement because of its unique health benefits (Berk, Malhi, Gray, & Dean, 2013; Du et al., 2019; Samuni, Goldstein, Dean, & Berk, 2013; Slattery et al., 2015). The broad health benefits of NAC, make it a supplement in high demand, especially for elderly people. Studies have shown that taking a daily dose of NAC (as high as 7 g) is beneficial for older patients (Shi & Puyo, 2020; SIu, BKh, Kopylev, Soodaeva, & Chuchalin, 2002). Delivering large doses of NAC in a palatable easy to use form, such as a ready-to-mix powder, would make it more desirable as a nutritional supplement.

Unfortunately, NAC in water is acidic (pH ~2) and has a strong and unpleasant aftertaste and a strong smell of rotten eggs due to the presence of a thiol group (–SH) thus, making it very unpleasant to drink a solution of NAC in water. There are, however, some NAC products on the market in the form of pills and gelatin capsules that can be taken without experiencing the unwanted aftertaste or smell. But, these products provide a maximum dosage of 1 g of NAC per serving, far short of the 7 g recommendation for daily use. A possible solution for introducing a high dose without the undesirable taste and smell is to coat NAC granules with hydrophobic food-safe materials that can provide a reasonable NAC release into the water before being consumed.

The health and pharmaceutical qualities of NAC along with its undesirable organoleptic properties make NAC an ideal model compound for new encapsulation technologies. The encapsulation of hydrophilic small molecules, specifically NAC, has been met with challenges (Aditya, Espinosa, & Norton, 2017; Dalmoro et al., 2017; McClements, 2015). While there are some successful reports of NAC encapsulation in the literature many include non-food safe materials and poor NAC loading. Examples of previous studies include using poly (lactic-co-glycolic acid) (PLGA) to encapsulate NAC via electrospray (Zarchi et al., 2015) with 5 wt % NAC loading. Another report includes creating a double emulsion (W/O/W) which increased NAC loading to 13.3 wt % (Murphy & Lampe, 2018). PLGA was also used for NAC and NAC salts whereby injectable implants, millicylinders, were prepared via solvent extrusion or fluid energy micronization with a maximum of 10 wt % loading (Desai, Mallery, & Schwendeman, 2008). PLGA was also used in combination with soybean lecithin to produce hybrid nanoparticles containing up to 38 wt % NAC (Ahmaditabar et al., 2017). Liposomal encapsulation of NAC using phospholipids via a reverse-phase evaporation method followed by spray drying has been reported to yield a 1 wt % NAC loading (Ourique et al., 2014). More recently, encapsulation of NAC in PEGylated nano-niosomes, vesicular nano-carriers composed of nonionic surfactants, with theoretical loading of 6 wt % has been reported for the treatment of acetaminophen poisoning (Span 60) (Firozian, Karami, Ranjbar, Azandaryani, & Nili-Ahmadabadi, 2020). Liposome-encapsulated NAC has also been prepared using the dehydration-rehydration method and NAC loaded liposomes have been prepared by a film hydration method and coated with chitosan for pulmonary delivery (Alarfaj et al., 2022; Buonocore et al., 2011; Hamedinasab, Rezayan, Mellat, Mashreghi, & Jaafari, 2020). With the maximum loading capacity for these previous reports less than 15%, a new encapsulation process using food grade or generally recognized as safe (GRAS) materials are needed.

Using the IPS process, we developed a phase separation process for the encapsulation of NAC. We used an inverse suspension process with food-safe and GRAS materials for a one-pot granulation and hydrophobic encapsulation of NAC using corn oil as a solvent. Spherical NAC/gum/EC granules (100–1000 μm in diameter) were produced with 56–78 wt % NAC loading. The NAC release profile from these encapsulated granules was studied using conductometry.

While only NAC was used for encapsulation in this work, our modified internal phase separation encapsulation process can be used to incorporate other nutrients and hydrophilic compounds as the solvents chosen to have the most impact on loading and encapsulation rather than the encapsulant. Thus, this process can be readily adapted to other hydrophilic small molecules and therefore this paper serves as a proof-of-concept for the encapsulation of water-soluble supplements for short-term delayed-release.

2. Experimental

2.1. Materials

NAC crystals were used as received from Nestle. Corn oil was purchased from a local market. hydroxypropyl methyl cellulose (HPMC) (40–60 mPa s, 2 wt % in water, Fisher), carboxymethyl cellulose sodium salt, CMC (low viscosity, 50–200 mPa s, 4 wt % in water, Sigma), CMC (high viscosity, 500–900 mPa s, 2 wt % in water, TCI America), Methyl cellulose, MC (1500 mPa s, 2 wt % in water, Sigma), casein sodium salt from bovine milk (Sigma-Aldrich), sorbitan monostearate, (Span 60, saponification value: 145 to 156, TCI America), sorbitan monooleate, (Span 80, saponification value: 145 to 160, Sigma-Aldrich), Dioctyl sulfosuccinate sodium salt (AOT, 96%, VWR), Polysorbate 80 (Tween 80, Sigma-Aldrich), soy lecithin (Archer Daniels Midland Company), ethyl cellulose (EC, 48.0–49.5% w/w ethoxyl basis, Sigma-Aldrich), carnauba wax (CW, melting point 82 °C, No.1 yellow, refined, Sigma-Aldrich), beeswax (BW, DR-101, melting point 62–65 °C, Strahl & Pitsch, Inc.), Candelilla wax (CanW, melting point 68–72 °C, Sigma-Aldrich), rice bran wax (RBW, melting point 79–85 °C, Nutley's Kitchen Gardens), acetonitrile (HPLC grade, ≥99.9%, Sigma-Aldrich), formic acid, (98%, Sigma-Aldrich), ethyl acetate (EtOAc, ≥99.5%, Certified ACS, Fisher), hexanes (≥98.5%, Certified ACS, Fisher), acetone (99.8%, Certified ACS, Fisher), and DMSO-d_6 (99.9%, Cambridge Isotopes, Inc.) were used in our experiments. Deionized (DI) water was used in all the experiments, and Milli-Q water was used for the analyses. All materials were used as received.

2.2. Characterization

Fourier transform infrared spectra (FTIR) of samples were obtained with a Shimadzu IRAffinity-1S spectrophotometer equipped with attenuated total reflectance (ATR) accessory. FTIR spectra of samples were measured from 400 to 4000 cm^{-1} with 64 scans at 2 cm^{-1} resolution. Nuclear Magnetic Resonance (NMR) Spectroscopy was carried out using a Bruker AV-500 NMR spectrometer at 23 °C. To prepare samples for ^1H and ^{13}C NMR spectroscopy, they were dispersed in DMSO-d_6 for 24 h, then filtered through 0.22 μm Nylon filters. Scanning electron microscopy (SEM) micrographs were prepared using a Zeiss Gemini 500 Field Emission SEM. An ultra-thin gold layer was applied to samples using a sputter coater to prepare them for SEM microscopy. The bright-field microscopy images of samples were taken by a LEICA DFC 3000 G microscope. Samples were put on a microscope slide and a water/glycerin solution (50:50 v/v) was added to wet them.

The kinetics of NAC release from samples were measured using conductivity. For this purpose, a Metrohm 856 Conductivity Module, equipped with a 5-ring probe electrode (0.7 constant, range of 5 uS/cm – 20 mS/cm) was used. A 100 mL beaker (4.5 cm ID and 7.5 cm length) was filled with 80 mL water, a stir bar (2.0 × 0.7 cm) was added, and the electrode was fully submerged in water. Pure NAC (2.00 g) or encapsulated samples were added to the beaker 60 s after starting the measurement and stirred at 500 rpm. The conductometric data was recorded for 600 s using Tiamo software Version 2.5. A calibration curve for conductivity vs. concentration of the NAC was prepared and used to measure the NAC release percentage of each sample 5 min after its addition to water.

To measure the NAC loading of samples, liquid chromatography (Agilent 1100 series) coupled with mass spectrometry (LCMS) was used. A Luna Omega (Phenomenex) liquid chromatography column (100 × 4.6 mm, 3 μm, Polar C18 100 Å) was used for reverse-phase chromatography at room temperature with two solutions (solution A: formic acid 0.1 v/v % in DI water and solution B: acetonitrile) in an isocratic elution (A to B ratio of 90:10 v/v). The flow rate and injection volumes were 0.3 mL min^{-1} and 10 μL, respectively. The mass spectrometry was performed using a Finnigan LTQ mass spectrometer with an electrospray interface (ESI) in positive electrospray ionization mode. For the measurements, sheath gas flow rate was set at 20 arbitrary units, aux gas flow rate at 0 arbitrary units, and sweep gas flow rate at 0 arbitrary units. Spray voltage was set at 4.00 kV, the capillary temperature at 350 °C, the capillary voltage at 41.0 V, and tube lens voltage at 125.0 V. To determine NAC loading in each sample, a portion of the samples was ground using a glass rod, then, 7.5 mg of each sample were dispersed in 15.0 mL of a 0.1 v/v % formic acid solution in DI water. Samples were placed on an Orbital Shaker for 24 h, after which they were filtered and analyzed via LCMS.

2.3. Granulation and encapsulation of NAC via internal phase separation (IPS) in one-pot

A concentrated solution of NAC, a gum, and a surfactant (either AOT or Tween80) in water was stirred at 70 °C for 2 h to ensure the dissolution of the gum. To this solution, a solution of EC in ethyl acetate (EtOAc) (10 wt %) was added and the mixture was either mixed thoroughly by a mechanical mixer or homogenized at 70 °C using a T25 digital Ultra-Turrax. The single-phase and almost transparent solution (Fig. 1) was transferred slowly to a 500 mL glass jacketed reactor that contained corn oil at 55 °C and stirred using a mechanical stirrer at 400 rpm. The mixture was allowed to stir overnight (15 h) at 55 °C, at which time the temperature was increased to 70 °C to help drive off the water/EtOAc from the NAC/gum/EC granules (Scheme 1). After the reaction finished, granulated samples were allowed to settle to the bottom of the reaction flask, the excess corn oil was decanted, and the remaining products were washed with cold hexane and vacuum filtered to dry. The specific conditions for the samples prepared by the IPS method are found in Table 1.

2.4. Coating and granulation of NAC in one-pot by conventional suspension process

To compare the samples produced by the IPS process with samples prepared by suspension coating, we prepared a few NAC granulated and coated particles by conventional suspension coating. An aqueous solution of NAC containing a hydrocolloid (CMC or HPMC) and AOT surfactant as structurant was prepared at 70 °C and mixed for 2 h to ensure complete dissolution of the gum. This solution was then added slowly to a solution of a wax and surfactant in corn oil at 70 °C in a 500 mL glass jacketed reactor with mechanical stirring at 400 rpm using a foldable paddle Teflon coated stirring shaft (Scheme 2). The temperature of the reactor was decreased to 55 °C and the reaction continued overnight (for 15 h) to allow slow evaporation of the aqueous phase. After 15 h at 55 °C, the temperature was then decreased to 25 °C, stirring was turned off and the granules were allowed to settle to the bottom of the flask. The excess oil was decanted, and samples were washed with hexane and vacuum filtered to dryness before analysis. The reaction conditions for the samples prepared with this method can be found in Table 2.

3. Results and discussions

3.1. NAC granulated/encapsulated samples prepared via IPS

Tables 1 and 2 show the composition and reaction conditions for the NAC granulated/encapsulated samples prepared by the IPS and conventional suspension methods, respectively. The water content of the dispersed phase is the minimum amount required to dissolve all of the core ingredients. This helps with faster removal of water over the course of the reaction. As is seen in Table 1, all of the IPS reactions were done using EC in EtOAc, because only the combination of NAC/gum/surfactant in water with EC in EtOAc can provide the one phase reaction mixture (Fig. 1) suitable for use as the dispersed phase in corn oil. This dispersed phase is then able to undergo phase separation during the reaction (Scheme 1). The boiling point of EtOAc (~77 °C) is well below that of water, therefore it started evaporating faster than water after the NAC/EC mixture was added to the corn oil at 55 °C. This triggered the phase separation process in the dispersed droplets (Scheme 1, step 1). Evaporation of EtOAc resulted in gradual precipitation of EC inside the droplets and, since EC is more hydrophobic than NAC and gum, it tends to migrate to the oil/water interface and form the shell/coating layer around the droplet (Scheme 1, step 2 and 3).

3.2. Morphology and size distribution of NAC granules

We used light microscopy and SEM to investigate the morphology, shape, and size of the granulated/encapsulated NAC particles prepared by the IPS method. The bright field light microscopy images were obtained after wetting the samples with a water/glycerin (50:50) solution (Fig. 2). The samples are spherical and most show a core-shell structure in the water/glycerin solution. We believe that the combination of the ethylcellulose hydrophobic shell, coupled with the dense core that consists of NAC/gum/surfactant, helps to retain the NAC inside the granules. The encapsulated particles showed a size distribution ranging from 100 to 1000 μm (Fig. 2 and Fig. S1). The particle size was determined by stirring method (homogenization versus mechanical stirring), stirring rate, and the viscosity of the aqueous phase.

The SEM micrographs of some of the samples prepared by the IPS method showed many NAC crystals on the surface of the granules, likely due to the high temperature used at the beginning of the granulation process (Fig. 3; Table 1, entry 3). The high temperature during the IPS process causes the fast removal of the water from the aqueous phase which brings some of the dissolved NAC with it to the surface. Performing the IPS step at lower temperatures ensured a slower rate of water removal and therefore led to less crystallized NAC on the surface (sample I3, Fig. 3b1, b2). The presence of NAC crystals on the surface of the granules, as well as some shell coating deficiencies, is most likely responsible for the initial fast release of the NAC observed in some samples.

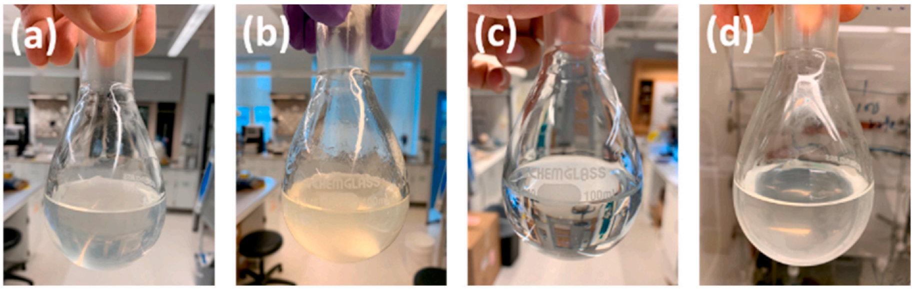
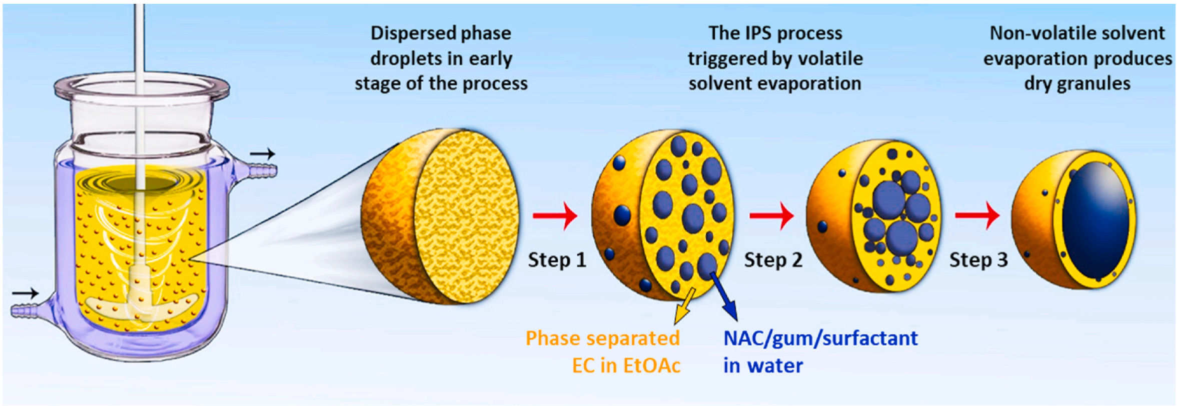


Fig. 1. The dispersed phase containing NAC/gum/surfactant in water and EC in EtOAc, is used in the IPS process. (a) sample I2, (b) sample I7, (c) sample I11, and (d) sample I15. See Table 1 for the reaction formulations and conditions.



Scheme 1. One-pot, one-step granulation/encapsulation of NAC via IPS.

Table 1
Formulations and reaction conditions for preparation of NAC encapsulated samples by IPS method.

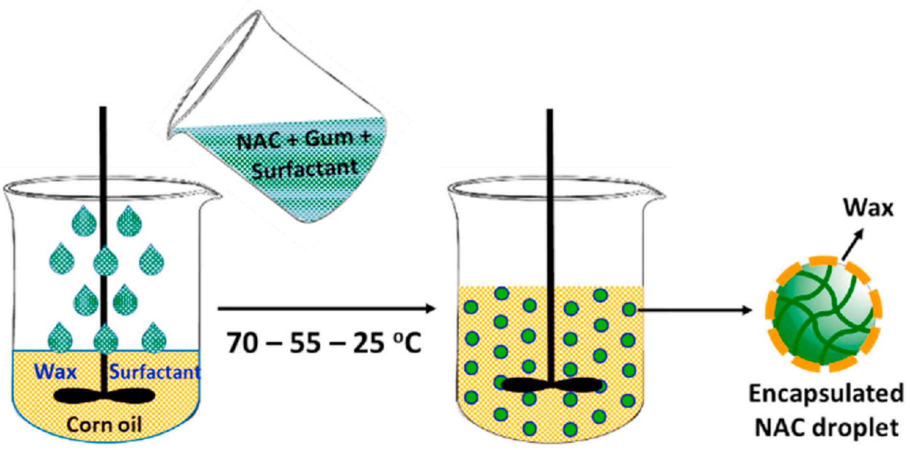
Sample code	Dispersed phase composition [weight percentage of each component]	Dispersed media Aqueous to Organic ratio (v/v)	Reaction temperature (°C) and time (h)	Theoretical vs. Experimental NAC loading (%)	Normalized NAC release (wt%) in 5 min
I0	[NAC + CMC + Tween80] [75:20:5]	Corn oil (1:10)	55 (15 h), 70 (2 h)	75.0/73.1 ± 0.8	95.7
I1	[NAC + Tween80] + [EC in EtOAc]; [95:5] + [10]	Corn oil (1:10)	55 (1 h), 60 (15 h)	86.4/63.1 ± 0.7	82.2
I2	[NAC + CMC + AOT] + [EC in EtOAc]; [75:20:5] + [10]	Corn oil (1:10)	70 (3 h), 55 (15 h)	68.2/56.3 ± 0.5	57.8
I3	[NAC + CMC + AOT] + [EC in EtOAc]; [75:20:5] + [10]	Corn oil (1:10)	55 (15 h), 70 (2 h)	68.2/70.8 ± 0.9	48.8
I4	[NAC + CMC[a] + AOT] + [EC in EtOAc]; [75:20:5] + [10]	Corn oil (1:10)	55 (15 h), 70 (2 h)	68.2/65.2 ± 0.2	60.5
I5	[NAC + CMC + AOT] + [EC in EtOAc]; [75:20:5] + [20]	Corn oil (1:10)	55 (15 h), 70 (2 h)	62.5/59.6 ± 0.9	40.7
I6	[NAC + CMC[a] + Tween80] + [EC in EtOAc]; [90:6:4] + [10]	Corn oil (1:10)	55 (15 h), 70 (2 h)	81.8/78.3 ± 0.6	69.1
I7	[NAC + CMC + Tween80] + [EC in EtOAc]; [72:23:5] + [20]	Corn oil (1:10) Span 80 (1.0 wt%)	55 (15 h), 70 (2 h)	60.0/63.1 ± 0.7	36.8
I8	[NAC + CMC + Tween80] + [EC in EtOAc]; [75:20:5] + [15]	Corn oil (1:10) Span 80 (1.0 wt%)	55 (15 h), 70 (2 h)	65.2/62.5 ± 2.3	31.9
I9	[NAC + HPMC + AOT] + [EC in EtOAc]; [80:15:5] + [10]	Corn oil (1:10)	55 (15 h), 70 (2 h)	72.7/75.1 ± 2.5	79.1
I10	[NAC + HPMC + AOT] + [EC in EtOAc]; [80:15:5] + [15]	Corn oil (1:10)	55 (15 h), 70 (2 h)	69.6/70.2 ± 1.7	71.0
I11	[NAC + HPMC + AOT] + [EC in EtOAc]; [75:20:5] + [10]	Corn oil (1:10) Span 80 (1.0 wt%)	55 (15 h), 70 (2 h)	68.2/71.7 ± 1.6	66.9
I12	[NAC + HPMC + AOT] + [EC in EtOAc][b]; [75:20:5] + [15]	Corn oil (1:10) Span 80 (1.0 wt%)	55 (15 h), 70 (2 h)	65.2/70.0 ± 4.9	53.3
I13	[NAC + HPMC + AOT] + [EC in EtOAc][b]; [75:20:5] + [20]	Corn oil (1:10) Span 80 (1.0 wt%)	55 (15 h), 70 (2 h)	62.5/65.4 ± 2.4	32.2
I14	[NAC + HPMC + AOT] + [EC in EtOAc]; [80:15:5] + [10]	Corn oil (1:10) Span 80 (1.0 wt%)	55 (15 h), 70 (2 h)	72.7/62.0 ± 0.7	45.0
I15	[NAC + HPMC + AOT] + [EC in EtOAc]; [75:20:5] + [20]	Corn oil (1:10)	55 (15 h), 70 (2 h)	62.5/64.7 ± 0.1	36.4

[a] High viscosity CMC.
[b] The dispersed phase was homogenized before addition to corn oil.

Fig. 4 shows the SEM micrographs of two samples (S1 and S4) prepared by the conventional suspension process. Sample S1 prepared using BW as the hydrophobic wax with NAC phase composition of [NAC + CMC + AOT]: [75:20:5], and sample S4 prepared using CW with NAC phase composition of [NAC + HPMC + AOT]: [75:20:5] (Table 2). Both samples have NAC crystals on their surfaces, but sample S4 shows fewer crystals and thus a better coating than sample S1. This observation was further confirmed by the NAC release data obtained through conductivity studies (Table 2).

3.3. *NAC loading and structural characterization of NAC granules by LC-MS, FTIR, and NMR*

Our goal was to obtain high NAC loading to minimize the uptake of other ingredients during a high dosage NAC therapy. Since NAC is cheap and can be extracted from natural sources, such as duck feathers, highly-loaded NAC granules are also cost-effective. We designed the formulations for the IPS process and calculated a theoretical NAC loading of 60 wt % or higher (Table 1). To determine the actual amount of NAC that was loaded into the final products, we measured the experimental loading (wt %) in the granules produced by the IPS process using LC-MS (Fig. S2). The NAC loading values for prepared samples ranged from 56



Scheme 2. Coating and granulation of NAC in one-pot suspension.

Table 2
Formulations and reaction conditions for NAC granules coated with waxes and prepared by suspension.

Sample code	Aqueous phase composition (wt%)	Organic phase composition (v/v for Aq. to Or. dispersion)	Reaction temp., °C and time (h)	Th. vs. Exp. NAC loading (%)	Normalized NAC release (wt%) in 5 min
S1	[NAC + CMC + AOT]: [75:20:5]	Corn oil (1:10), BW (20) Span 60 (1.0 wt%)	70 (1 h), 55 (15 h)	62.5/ 65.8 ± 0.8	58.5
S2	[NAC + CMC + AOT]: [75:20:5]	Corn oil (1:10), CW (15) Span 60 (1.0 wt%)	70 (1 h), 55 (15 h)	65.2/ 67.2 ± 1.2	54.1
S3	[NAC + HPMC + AOT]: [75:20:5]	Corn oil (1:10), BW (20) Span 60 (1.0 wt%)	70 (1 h), 55 (15 h)	62.5/ 63.2 ± 1.3	56.3
S4	[NAC + HPMC + AOT]: [75:20:5]	Corn oil (1:10), CW (15) Span 60 (1.0 wt%)	70 (1 h), 55 (15 h)	65.2/ 70.4 ± 1.7	50.4

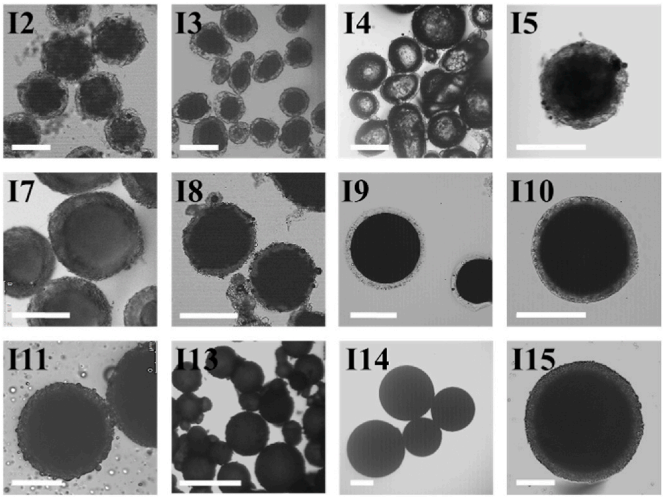


Fig. 2. Bright-field microscopy images of the NAC granulated/encapsulated samples prepared by the IPS method (scale bar = 200 μm). See Table 1 for the reaction formulations and conditions.

to 78 wt %. Table 1 shows the theoretical and experimental NAC loadings of all samples. We found that the experimental NAC loadings for the IPS samples were close to the theoretical values in most cases. Since we used selected reaction monitoring (SRM) for our LC-MS measurements, the values obtained are for the NAC itself that prove the stability of NAC during the IPS encapsulation condition.

Fig. 5 shows the ATR-FTIR spectra of pure NAC and some of the representative samples prepared in the IPS process. The FTIR spectra of the starting materials used for the IPS process are shown in Fig. S3. The pure NAC characteristic bands appeared at 3370 cm^{-1} due to the presence of secondary N–H stretching vibrations (Pavia, Lampman, Kriz, & Vyvyan, 2008), and at 2550 cm^{-1} as a result of the free S–H stretching (Du et al., 2019; Pathan, Solanki, & Patel, 2017; Pavia et al., 2008). Further, the band at 1715 cm^{-1}, related to the carbonyl group vibrations (Hamedinasab et al., 2020), and at 1530 cm^{-1} resulting from the N–H bending (Pavia et al., 2008), along with the stretching frequency of the carboxyl groups at 535 cm^{-1} prove the existence of NAC inside the capsules (Fig. 4 and Fig. S4) (Du et al., 2019). The encapsulation of NAC was also confirmed by the NMR and LC-MS data. However, there were some additional bands observed, especially at 2840–3000 cm^{-1}, that we attributed to the aliphatic C–H bond stretching of CMC, HPMC, or the EC coatings (Pavia et al., 2008). Also, the carbonyl band is broad with a shoulder on the left side in all the samples prepared by either IPS or suspension processes, which indicates the presence of an extra carbonyl bond from the gum, or the interaction of some of the NAC molecules with the gum that has caused the shift in carbonyl band absorbance. The FTIR of samples prepared by the conventional suspension process were compared with pure NAC, and the analysis of the spectra confirmed the presence of NAC and the wax in all of the samples (Fig. S4).

The LC-MS and FTIR results showed the presence of NAC in all samples. To further confirm the presence of NAC inside the granules, and its structural integrity after IPS processing, we used ^{1}H and ^{13}C NMR spectroscopy. Two representative samples (I8 and I15) were shaken in DMSO-d_6 for 24 h and filtered. Then, their NMR spectra were recorded and compared to the spectrum of pure NAC in DMSO-d_6 (Sisombath & Jalilehvand, 2015). Comparison of these spectra with that of pure NAC confirmed the presence of NAC inside the capsules (Figs. S5 and S6). Further, the LC-MS and NMR results confirmed that the structure of the NAC was stable and did not change during the lengthy EtOAc and water removal phase of the IPS process (Sisombath et al., 2015).

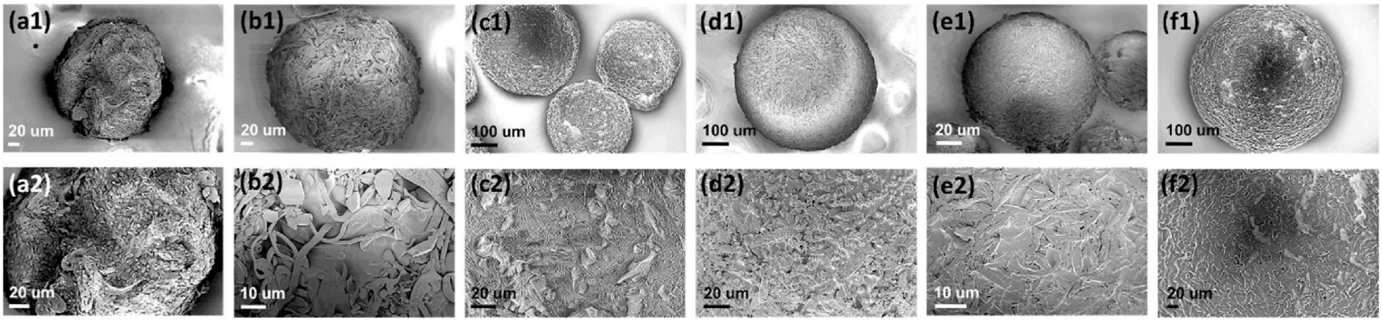


Fig. 3. SEM micrographs of the NAC granulated/encapsulated samples prepared by the IPS method with two magnifications, row two are magnifications of the particles in row one. (a1-a2): sample I2, (b1-b2): sample I3, (c1-c2): sample I7, (d1-d2): sample I9, (e1-e2): sample I13, and (f1-f2): sample I15. See Table 1 for the reaction formulations and conditions.

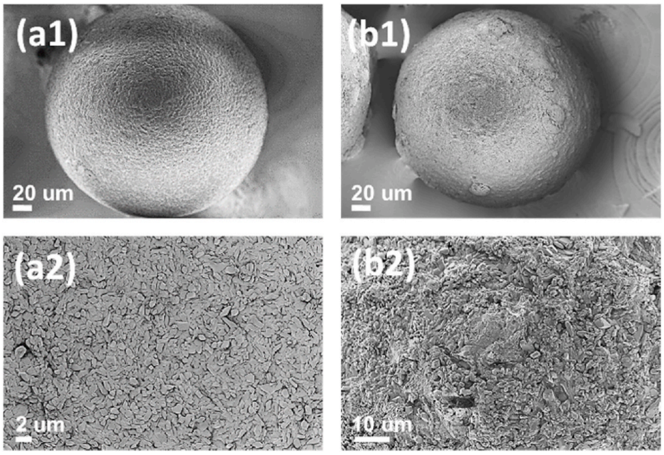


Fig. 4. SEM micrographs of the NAC granulated/coated samples prepared by the suspension process with two magnifications, row two is a magnification of the particles in row 1. (a1-a2): sample S1 and (b1-b2): sample S4. See Table 2 for the reaction formulations and conditions.

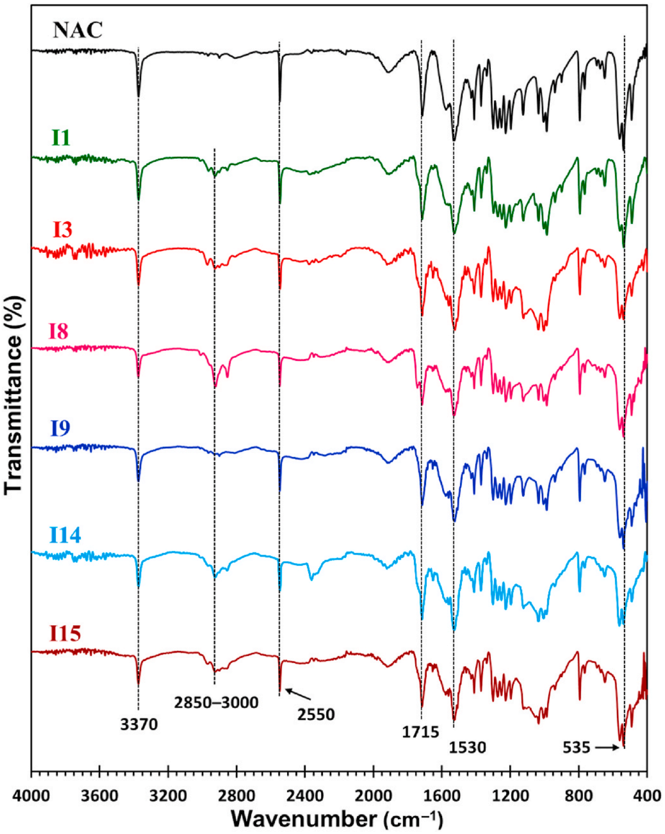


Fig. 5. ATR-FTIR of pure NAC and some of the representative samples prepared via IPS process. See Table 1 for the reaction formulations and conditions.

3.4. *Conductometric study of NAC release in water*

Conductometry is a powerful tool for measuring the concentration and release of compounds from microcapsules. NAC solutions exhibit high conductivity, so NAC concentrations of tablets and other products have been measured using conductometry (Janegitz, Suarez, Fatibello-Filho, & Marcolino-Junior, 2008; Rizk, Belal, & Eid, 1993; Santos, Guerreiro, Suarez, Faria, & Fatibello-Filho, 2011). We used conductometric measurements to determine the rate of NAC release from the IPS and suspension coated samples. Plotting conductivity vs. time allowed us to determine the kinetics of NAC release as conductivity is directly proportional to NAC concentration. Fig. 6 shows the produced graphs of conductivity vs. time for all the IPS samples prepared with either CMC (Fig. 6a) or HPMC (Fig. 6b) as the gum. The amount of pure NAC and the IPS samples are calculated that for all of the samples a total of 2.5 wt % NAC in water is provided (i.e. 2 g total available NAC in 80 mL DI water).

These release profiles resemble those of other encapsulated hydrophilic small molecules, such as ascorbic acid and potassium chloride (Giannola, De Caro, & Severino, 1995; Harris, 1981; Navarro-Guajardo et al., 2018; Uddin, Hawlader, & Zhu, 2001). Encapsulation of highly hydrophilic materials and the prevention of their fast release into the water, especially in products with high water activity, has been a longstanding challenge (Aditya et al., 2017; Dalmoro et al., 2017; McClements, 2015). NAC is a small hydrophilic molecule (MW = 163.19 g mol^{-1}) with a water solubility of 20 wt % at 25 °C, and a very fast dissolution profile. The conductivity of the pure NAC solution reached the maximum in just a few seconds after its addition to water (Fig. 6). Consequently, keeping NAC away from water molecules is a big challenge, even with encapsulation. All samples prepared by the IPS process, except the control sample I0, show a delayed release of NAC (Fig. 6). Sample I0 was prepared in the absence of hydrophobic EC (Table 1) offers no barrier for NAC dissolution in water, and releases ~96 wt % of its NAC cargo in 5 min after addition to water. Sample I1, which was prepared using EC as the hydrophobic coating but without any gums (Table 1, entry 2), shows a better NAC retention than I0 and releases around 82 wt % of the NAC cargo in 5 min. Comparing these results with the NAC release results of other samples in Table 1, we observe that the combination of two gums (CMC and EC) have a synergistic effect on the prevention of premature NAC release by providing a more robust network of entangled polymers. All the IPS samples containing CMC or HPMC, and EC as the coating, showed moderate NAC

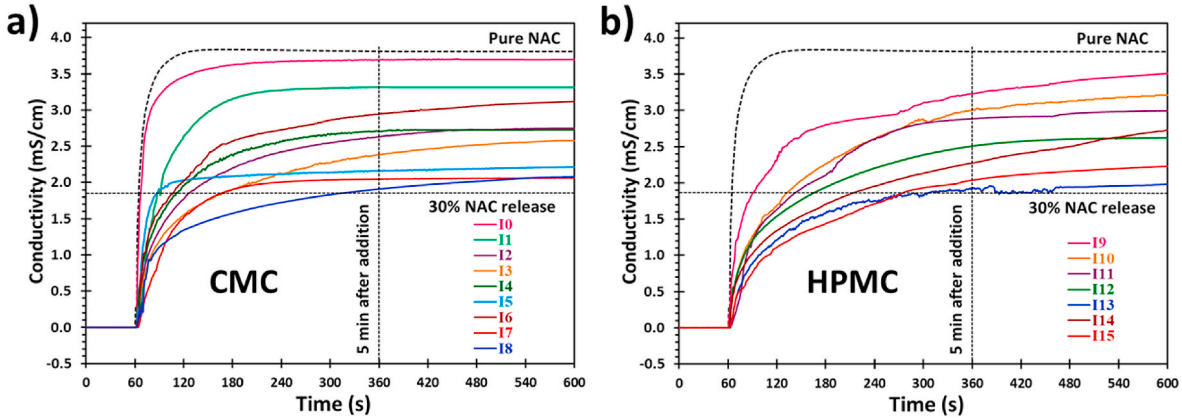


Fig. 6. The release profiles (conductivity vs. time) of NAC granulated samples were prepared by the IPS process. a) samples prepared with CMC, b) samples prepared with HPMC (all samples were added at 60 s). See Table 1 for the reaction formulations and conditions.

retention and release between 32 and 79 wt % NAC within 5 min (Table 1). Fig. S7 shows the images of the NAC release form sample I8 during a period of 50 min under a microscope. It is obvious from these images that the NAC/gum/surfactant core gradually dissolved and was washed away by water.

The initial NAC release profiles, as observed using conductivity for samples prepared via a simple suspension using beeswax and carnauba wax, are slightly different from samples prepared via internal phase separation. However, 5 min after addition, they all show more than 50 wt % NAC release (Fig. 7). These data are in agreement with the SEM micrographs for these samples as well (Fig. 4). For all of these samples, the experimental NAC loading is higher than the theoretical loading, which means that not all of the wax in the formulation has been used for coating the NAC granules.

For an IPS process to perform successfully, it is important to control the temperature profile. High temperatures during the IPS granulation and encapsulation process can lead to fast removal of both solvent and uncontrolled processes. Especially, the fast removal of the water can result in "leaking" of the NAC on the surface of the particles. To study the effect of temperature during the granulation/encapsulation process, samples I2 and I3 were prepared (Table 1 entries 3 and 4). They are both prepared with the same composition: [NAC + CMC + AOT] + [EC in EtOAc] = [75:20:5] + [10], with different temperature profile. Sample I2 has been heated at 70 °C for 3 h followed by 15 h heating at 55 °C, while sample I3 has been heated at 55 °C for 15 h followed by 2 h heating at 70 °C. The SEM images (Fig. 3a1, a2) of sample I2 shows evidence of substantial NAC crystals on the surface, while I3 which was made at lower granulation and coating temperatures shows fewer crystals on the surface (Fig. 3b1, b2). This was confirmed by the higher conductivity of sample I2 that revealed higher NAC release in 5 min than sample I3 (57.8% vs. 48.8%, Table 1 and Fig. 6).

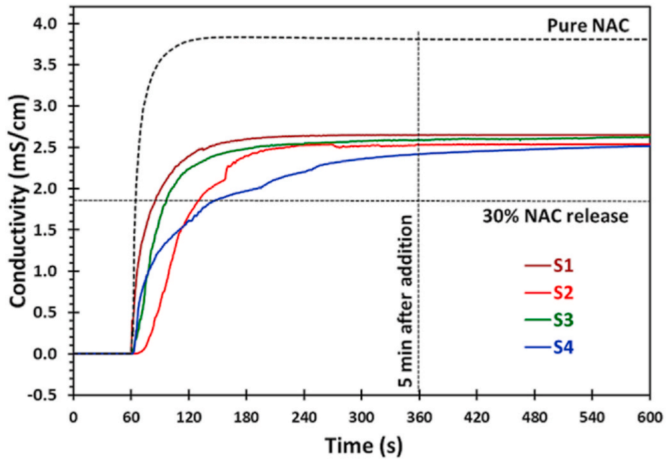


Fig. 7. The conductivity vs. time for the NAC granulated samples prepared via suspension (all samples were added at 60 s). See Table 2 for the reaction formulations and conditions.

In a ready-to-mix product application, the additive must be easily dissolved or dispersed in water before consumption, thus the choice of an appropriate coating becomes important. Therefore, a simple dispersion of NAC-containing granules into water is desirable. To achieve this, a moderately hydrophobic coating is best as a superhydrophobic coating would lead to an agglomeration of the particles upon addition to the water. In some cases, an extra coating (a thin layer of hydrophilic gum or sugar) can be used on top of a hydrophobic material to improve the product's dispersibility in water (Kommineni, Ahmad, Vengala, & Subramanyam, 2012; Sakurai et al., 2017). However, EC has moderate hydrophobicity and thus all of the samples that were prepared by the IPS process showed desirable dispersibility in water (Fig. S8).

We observed that increasing the EC content in the same formulation decreased the NAC release (in Table 1, compare samples I3 with I5 and samples I11, I12, and I13), as it is the hydrophobic component that delays the water access to the granule core (Oshlack, Chasin, & Pedi, 2008; Rane, Gattani, Kadam, & Tekade, 2009). As for the effect of gum, while both CMC and HPMC provide acceptable NAC release, the HPMC formulation can provide a slower initial NAC release (Fig. 6).

4. Conclusions

We have effectively shown that IPS can be used to granulate and encapsulate NAC (at high loading levels) from food safe and GRAS materials by a phase separation of a dispersed mixture consisting of two solvents, where one solvent evaporates faster during the granulation/encapsulation process. Subsequent evaporation of water from the dispersed phase produced spherical granules containing 56 to 78 wt % NAC coated by the hydrophobic component (EC). Two gums, CMC and HPMC (as structurant), and four surfactants including AOT, Tween 80, Span 80, and Span 60 were used. All of the samples with gum and EC showed moderately delayed NAC release compared to the pure NAC in the water. Microscopy and SEM images showed core-shell structures and spherical granules with diameters ranging from 100 to 1000 μm. Conductometry revealed moderate retention of the NAC as low as 32 wt % release for some of the samples 5 min after addition to water. Moreover, they showed that both CMC and HPMC, in combination with EC as hydrophobic coating, provide a good barrier in NAC release. These high loading granulated/encapsulated NAC particles were made by a one-pot, one-step process, all GRAS materials, and are potential candidates for use as a ready-to-mix supplement for oral high dose NAC therapy. The

IPS method described here can be used for other hydrophilic supplements/ingredients for temporary delayed release in the aqueous media.

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Credit author statement

M. E: Conceptualization, Methodology, Investigation, Data curation, Writing - original draft, Writing - review & editing. **S. M:** Conceptualization, Investigation, Data curation, Writing - original draft, Writing - review & editing. **B. Y.:** Data curation, Resources, Writing-review & editing. **G. U.:** Supervision, Resources, Writing-review & editing. **A. A.:** Conceptualization, Supervision, Project administration, Funding acquisition, Resources, Writing – review & editing.

Declaration of competing interest

The authors declare no competing financial interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodhyd.2022.107699.

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