

Original Research Article

Modified extrusion-spheronization as a technique of microencapsulation for stabilization of choline bitartrate using hydrogenated soya bean oil

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Abstract

Introduction: Choline bitartrate (CBT) is a vital nutrient for fetal brain development and memory function. It is hygroscopic in nature which is associated with stability related problem during storage such as development of fishy odor and discoloration. **Aim:** Microencapsulation method was adopted to resolve the stability problem and for this hydrogenated soya bean oil (HSO) was used as encapsulating agent. **Materials and Methods:** Industrially feasible modified extrusion-spheronization technique was selected for microencapsulation. HSO was used as encapsulating agent, hydroxypropyl methyl cellulose E5/E15 as binder and microcrystalline cellulose as spheronization aid. Formulated pellets were evaluated for parameters such as flow property, morphological characteristics, hardness-friability index (HFI), drug content, encapsulation efficiency, and *in vitro* drug release. The optimized formulations were also characterized for particle size (by laser diffractometry), differential scanning calorimetry, powder X-ray diffractometry (PXRD), Fourier transform infrared spectroscopy, and scanning electron microscopy. **Results and Discussions:** The results from the study showed that coating of 90% and 60% CBT was successful with respect to all desired evaluation parameters. Optimized formulation was kept for 6 months stability study as per ICH guidelines, and there was no change in color, moisture content, drug content, and no fishy odor was observed. **Conclusion:** Microencapsulated pellets of CBT using HSO as encapsulating agent were developed using modified extrusion spheronization technique. Optimized formulations, CBT 90% (F5), and CBT 60% (F10), were found to be stable for 4M and 6M, respectively, at accelerated conditions.

Key words: Choline bitartrate, hydrogenated soya bean oil, jacketed spheronizer, microencapsulation

INTRODUCTION

Choline is a water-soluble essential nutrient and important for normal functioning of cells. It is well-recognized vital nutrient for fetal brain development and memory function, having a permanent impact on cognition.^[1,2] It is important for maintaining normal cell membrane formation, transmembrane signaling, cholinergic neurotransmission,

and lipid metabolism.^[3] It exists in free and esterified forms like choline bitartrate (CBT) salt. Literature reports that CBT is stabilized by encapsulation and thus protects choline against conditions encountered during manufacturing and storage of dosage form.^[4,5] CBT is a white crystalline low hygroscopic choline salt. Chemically, it is (2-hydroxyethyl) trimethylammonium - L - (+) - tartrate salt.^[6]

Choline bitartrate can be administered in the form of a tablet or capsule. The interaction of CBT with heavy metals present in multivitamin and mineral tablet like Gesticare[®] DHA generally destabilize it and leads to loss of stability of choline, decrease in its activity, odor development or discoloration of tablet during storage. This reduces the shelf life of choline. Therefore, there is a need to encapsulate choline salt which provide choline a desired degree of protection.^[7,8] Various lipids such as phospholipids, triacylglycerols, waxes, fatty acids or mixtures of them can be used for encapsulation.^[9] Waxes like hydrogenated soya bean oil (HSO) was used for microencapsulation of nutraceuticals such as tocopherol and iron.^[9,10] HSO has been used for coating which gives better protection and retardation of drug release due to their hydrophobic nature compared to hydrophilic polymers.^[8]

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But, its use has been restricted because it requires nonaqueous and hot-melt techniques for the formulation development. In the current study, CBT is microencapsulated with a lipid wax, hydrogenated vegetable oil (Soya bean oil). Microencapsulation is a creation of a barrier around the core material to avoid chemical reaction and to reduce the reactivity of the core in relation to the outside environment.^[11] These coating techniques often require a solvent, that is, water, organic solvent or mixture. The use of organic solvent is associated with the environmental problem, solvent residue, and excessive cost of recovery, whereas aqueous solvent generally prolongs the duration of coating process.^[12] The extrusion-spheronization technique study is utilized for encapsulation, which completely surrounds the core material with wall material.^[13] The main objective of the current study was to investigate the use of waxes such as HSO as encapsulating agent to stabilize CBT using extrusion-spheronization technique.

MATERIALS AND METHODS

Materials

Choline bitartrate and HSO were obtained as a gift sample from Bajaj Healthcare Ltd., Mumbai, India. Hydroxypropyl methyl cellulose (HPMC) (Methocel® E5 and E15) were procured from the Dow Chemical Co., USA. Avicel PH 101, 102 and lactose was procured from signet chemicals, Mumbai, India. Tween 80 was obtained from SD fine chemicals, Mumbai, India. All other chemicals used were of laboratory and reagents grade.

Methods

Fabrication of jacketed extrusion-spheronizer

A modification was made in the existing spheronizer (S.B. Panchal Ltd., Mumbai). A heater was attached to the circumference of the barrel. The heater was placed in such way that it is above the die plate and uniformly supplies the heat through conduction. The heating was controlled with temperature controller.

Formulation and development

Selection of spheronizing aid for pellets

The pellets using spheronizing aid like MCC PH 101, MCC PH102, and lactose were prepared by extrusion spheronization technique. The pelletization process and appearance of pellets were observed and evaluated.

Drug excipient compatibility study

Drug-excipient interaction study was performed using differential scanning calorimetry (DSC) analysis. DSC thermogram was

recorded for CBT, mixtures of the drug; Excipients. The pure drug, drug polymer mixture (1: 1), mixture of polymers (1: 1) were kept in the dried glass vial under normal conditions at room temperature for 15 days and these samples were analyzed on DSC, Perkin Elmer pyris-6 (USA) equipped with a thermal analysis computerized data station. Samples (3-4 mg) were heated in the aluminum pan at a rate of 10°C/min in 40-300°C temperature range under nitrogen flow of 20 ml/min using an empty sealed pan as reference.

Preparation of choline bitartrate extrudate and pellets

Choline bitartrate was used as a core material and HSO as a coating agent which coat the drug. CBT and MCC PH 101 were weighed accurately, sieved through 30 mesh sieve and mixed with the melted HSO at 70°C containing Tween 80 as plasticizer, butylated hydroxyl toluene as antioxidant, lemon oil as flavor in a Hobart mixer for a period of 10 min. The obtained blend was granulated with a binder solution (2% w/v HPMC E5 or E15) prepared in purified water to form a wet mass. This wet mass was then extruded through single screw extruder having 0.8 mm extruder screen. Extrudes were then spheronized using hot jacketed spheronizer which was maintained at a temperature of 60-75°C; equipped with 4.2 mm friction plate at 1200 rpm for 10 min (single screw extruder and fabricated hot jacketed spheronizer, S.B. Panchal, Mumbai). Melting of HSO at 60-65°C in a hot jacketed spheronizer gives complete covering of wax to the core material. After spheronization process, the obtained pellets were kept for drying in hot air oven.

Optimization of processing variables for extrusion-jacketed spheronization process

To formulate a pellet with desired characteristics (physical appearance and sphericity), the parameters such as speed of spheronization, temperature of jacketed spheronizer, and time of spheronization were varied in the range as mentioned in Table 1.

The different trials for wax coating were conducted and based on these trials the preliminary processing variables for wax coating were selected.

Formulation optimizations

The composition of the wax coating solution and other excipients should be selected such that obtained pellets have desired physical characteristics, good encapsulation efficiency, drug content, and flow properties. Pellets prepared without binder (i.e., only with

Table 1: Optimization of processing variables for extrusion-jacketed spheronization process

Process parameters	Conditions									
	For CBT 90%					For CBT 60%				
	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
Speed of spheronization	500	800	1000	1200	1400	500	800	1000	1200	1400
Time of spheronization	5	15	10	15	20	5	5	10	15	20
Temperature of jacketed spheronizer (°C)	60-65	65-70	70-75	70-75	70-75	55-60	60-65	60-65	65-70	70-75

CBT: Choline bitartrate

wax) and with binder HPMC E5 or E15 with wax were observed for their consistency and strength. Among used binders, HPMC E15 cps was chosen on the basis of their good binding capacity. Pellets obtained with HPMC 15 cps showed good compressibility index, hardness-friability index (HFI), and flow properties as compared to other binders. Different formulation compositions are shown in Table 2.

Evaluation of pellets

Morphological characteristics and flow properties

The size distribution in terms of average diameter of the pellets was determined by an optical microscopic method. A compound microscope fitted with a calibrated ocular diameter and stage micrometer slide was used to count at least 100 particles (Olympus, NWF 10x; Educational Scientific Stores, India).^[14] All the batches were studied with regards to the morphological features such as aspect ratio, particle size and shape using photomicrograph. The prepared pellets were also evaluated for the parameters like bulk density, tapped density, compressibility index (Carr's index), angle of repose, and hausner's ratio.

Hardness-friability index (%)

Hardness-friability index was calculated on the basis of the results of the friability test. 10 g of pellets were placed in a roche friabilator and rotated for 10 min at 25 rpm. The pellets were then screened through a 40 mesh sieve to remove the fines generated.

^[13] The HFI was calculated as follows:

$$\%HFI = F_b/F_a \times 100 \quad (1)$$

Where, F_b and F_a are weights before and after friability treatment, respectively.

Drug content

Analysis was performed as per method described in USP 30. Accurately weighed (200 mg) microcapsules of CBT were transferred to a conical flask and dissolved in 50 ml glacial acetic acid. Sample was titrated with 0.1 N perchloric acid, and endpoint was determined by potentiometrically (USP 30).

Table 2: Formulation composition for different batches

Formulation (%)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
CBT	90	90	90	90	90	60	60	60	60	60
HSO	8	7	5	5	5	28	23	19	19	19
HPMC E5	—	—	1	—	—	—	—	8	—	—
HPMC E15	—	—	—	1	1.5	—	—	—	8	9.4
MCC PH101	2.7	3.7	3.5	3.5	3	9.7	14.7	10.7	10.7	9.3
Tween 80	0.3	0.3	0.3	0.3	0.3	2	2	2	2	2
BHT	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Lemon flavour	0.2	0.2	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.2
Distilled water	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

CBT: Choline bitartrate, HSO: Hydrogenated soya bean oil, BHT: Butylated hydroxytoluene, MCC: Microcrystalline cellulose, HPMC: Hydroxypropyl methyl cellulose, q.s.: Quantity sufficient

For the determination of drug content factor given in USP was used.^[15]

Each ml of 0.1 N perchloric acid is equivalent to 25.32 mg of $C_9H_{19}NO_7$ (2)

Drug encapsulation efficiency

The encapsulated micro particles equivalent to 500 mg of CBT were accurately weighed and crushed. The powdered microparticles were dissolved in dichloromethane (5 ml) in volumetric flask and made the volume with 0.1 N HCl. The solution was then filtered through whatmann filter paper No. 44. After suitable dilution, the absorbance was measured at 208 nm using ultraviolet spectrophotometer and the percentage drug entrapped was calculated (equation 2). The drug entrapment study was conducted in triplicate.^[14]

$$\% \text{ Drug encapsulation efficiency} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \quad (3)$$

Moisture uptake and stability studies

Moisture uptake by drug and encapsulated drug was studied using moisture balance MB 50C (Citizen, India). After moisture content analysis, of CBT and encapsulated CBT were placed in crucible at accelerated conditions of temperature $40^\circ \pm 2^\circ\text{C}$ and humidity $75\% \pm 5\%$ relative humidity (RH) in environmental test chamber for 24 h (Thermo lab, India). These samples were then analyzed for drug content by potentiometric titration. This method is useful to determine the effect of moisture on degradation of the drug and encapsulated drug systems.^[16,17]

In vitro drug release

The 5 h release of encapsulated CBT in water was determined as 1 g of CBT was placed in 250 ml of elementary flask containing 100 ml of distilled water and flask was sealed with a stopper. The flask was then placed on a shaker for 5 h with moderate shaking intensity. At the end of 5 h, content of the flask was then filtered through premoistened glass wool in a powder funnel. Elute was then collected in second flask. The first flask was then rinsed with 10 ml of water and added to the second flask through powder funnel. The obtained solution was then titrated with 0.1 N NaOH. The end point of the titration was determined by measuring the first inflexion in the pH values, at approximately neutral pH. The percent drug release was calculated using the following equation:^[8]

$$\text{Percent Release} = \frac{\text{ml of 0.1 N NaOH} \times 0.1 \times 253 \times 100}{\text{mg of Encapsulate} \times (\% \text{ of CBT} / 100)} \quad (4)$$

Solid state characterization of optimized pellets formulation

Particle size analysis

The optimized pellets were evaluated for their particle size distribution using the laser light diffraction instrument (Malvern Instruments, Mastersizer 2000 Ver. 5.30.010, UK). The pellets were fed into the sample micro feeder. All samples were analyzed 5 times, and average results were taken. The characterization of

pellet size distribution was analyzed by below 10% d (0.1), 50% d (0.5) and 90% d (0.9) of the pellets size. The average of d (0.1), d (0.5), and d (0.9) values is taken as mean diameter.

The size uniformity and dispersity of the microspheres were represented by span value. The narrow size distribution was indicated by small span value. Span value was measured from the following equation:^[18]

$$\text{Span} = (D90-D10)/D50 \quad (5)$$

Differential scanning calorimetry

Differential scanning calorimetry analysis was performed using Perkin Elmer DSC Pyris-6 (USA) on 4-8 mg sample. Samples were heated in the aluminum pan at a rate of 10°C/min in 40-240°C temperature range under nitrogen flow of 20 mL/min using an empty sealed pan as a reference. The obtained spectra were then studied for the interaction between drug and the other excipients of the encapsulated pellets.

Powder X-ray diffractometry

X-ray diffraction patterns were recorded on Rigaku Miniflex instrument for polymers using Ni filtered, Cu K_α as source of radiation, a voltage of 40 Kv, and a 25 mA current. The samples were run over the most informative range from 5° to 50° of 2θ values. The step scan mode was performed with a step size of 0.02° at a rate of 2°/min. Samples were ground into powder with mortar and pestle and measured on a low background quartz plate in an aluminum holder.

Fourier transforms infrared spectroscopy

Fourier transform infrared (FT-IR) spectrometer, PerkinElmer (USA) was used in an attenuated total reflectance manner to obtain FT-IR spectra. The samples were ground thoroughly with potassium bromide at 1: 100 (sample/potassium bromide) weight ratio in a mortar and pestle till a uniform mixture observed. Scans were performed in triplicate from 4000 to 400 cm⁻¹, with a threshold of 1.303, sensitivity of 50, and resolution of 2 cm⁻¹ range were recorded on KBr disc. Spectra were recorded for CBT powder and optimized pellets of CBT.

Scanning electron microscopy

To evaluate any changes in the physical surface, morphology of pellets like size and shape was analyzed using scanning electron microscope (XL 30 Model, JEOL 5400, Japan). Sample was prepared by affixing double-sided carbon tape on aluminum stubs over which sample of CBT and prepared pellet were placed. The radiation of the platinum plasma beam using JFC-1600 auto fine coater was targeted on aluminum stubs for its coating to make a layer of 2 nm thickness above the sample for 25 min. These prepared coated stubs were then placed in the vacuum chamber of a scanning electron microscopy (SEM) and adjusted to maximum magnification to obtain excellent quality scanning images. Then, those samples were observed for morphological characterization using a gaseous secondary electron detector (working pressure: 0.8 Torr, acceleration voltage: 20.00 kV). SEM

images were obtained at a maximum and visible magnification to understand the surface morphology between drug and wax.

Stability study

Stability study was conducted by placing samples in closed glass ambered color vials which were stored in a controlled temperature environment inside stability chamber with RH of 75% and 40°C temperature^[19] and 65% and 30°C temperature. Samples were reevaluated after 1, 2, 3, 4, 5, and 6M for physical appearance, discoloration, fishy odor development resembling amines, moisture content, and drug content.

RESULTS AND DISCUSSION

Hydrogenated soya bean oil has low melting point so when it is used in other techniques such as fluidized bed processor or spray drying the material got stuck to the expansion chamber during drying process and the nozzle gun also blocked during coating process. These techniques often require solvent which prolongs the duration of coating process.^[20-22] Fabrication of laboratory scale jacketed spheronizer was done which coats the core material with melting of wax in a jacketed spheronizer. Furthermore, the solvent required in this technique is very less as compared to other techniques.

Formulation development

Selection of spheronizing aid

The plasticity of damp mass and surface morphology of extrudate was considered as the key points for the selection of the spheronizing aid. Extrudate prepared with lactose shows good extrusion ability but did not have desired plasticity and surface appearance. MCC has better spheronizing capacity than lactose.^[23,24] But, the extrudate prepared with MCC PH 102 showed cracks with a granular surface which did not observed in case of MCC PH 101. Hence, MCC PH 101 was selected as spheronizing aid for further study.

Drug excipient compatibility study

The possible interaction between CBT with HSO and polymers was studied. There was no significant change in DSC endothermic values of plain CBT as compared to microencapsulated CBT. The peak value obtained at 155.15°C, which is corresponding to melting of plain drug. There was no major shifting in this peak value observed. Hence, it was concluded that there was chemical compatibility between drug and excipients. The DSC thermogram is shown in Figure 1a and b.

Optimization of processing variables for Extrusion-Jacketed spheronization Technique

Different trials were taken as mentioned in Table 1 for selection of processing variables for extrusion-spheronization technique. In T1 and T2 trial batch formulated with 90% CBT, the surface of the pellets was found to be very rough, and particle size was very irregular. This result was because of lower spheronization

speed at 60-70°C temperature of jacketed spheronizer. Similarly, at higher spheronization speed pellets get agglomerated due to melting of HSO at higher temperature. For the development of CBT, 90% and 60% wax coating batch T3 and T8 processing variables were taken into consideration.

Evaluation of pellets

Morphological characteristics and flow properties

The flow properties of all batches are shown in Table 3, which shows that plain CBT has very poor flow property whereas prepared formulations showed improvement in flow property. But, compressibility and binding capacity of pellets were relatively low therefore addition of the binder required to be added in formulation composition. Formulation F5 and F10 showed improvement of compressibility and binding of pellet as compared to other batches.

Assay of CBT was determined by potentiometric titration method.^[16] Table 3 shows that content in batch F1-F5 preparation were in the range of 89-91% and in case of batch F6-F10 it was in the range of 61.5-62.5% which implied that microencapsulation could produce good reproducibility of assay content. The encapsulation efficiency of microparticle varied between 61%

and 70% [Table 3]. Results demonstrate that increase in coating level increases encapsulation efficiency of the drug.^[14]

The morphological study of plain CBT showed that the irregular and nonuniform shape of particles [Figure 2]. The aspect ratio of pellets varied from 1.92 to 1.21 which indicate almost spherical shape of pellets while the F5 (90%) and F10 (60%) have aspect ratio of 1.08 and 1.02 with a more spherical and uniform pellets as compared to other batches.

Hardness-friability index

The average % hardness of all batches was in the range of 94-99%. The % HFI of batch F5 (90%) and F10 (60%) was 99.1% and 99.04%, respectively [Figure 3], which indicated that increasing in binder concentration increases the % HFI of the formulation.^[13]

Moisture uptake and stability studies

Moisture uptake study was conducted to check hygroscopic nature of the prepared pellets. No significant change in weight was observed after subjecting the sample to accelerated conditions of temperature and humidity.^[16,17]

Hygroscopic nature of CBT inside the encapsulated formulation and pure CBT was useful to understand the degradation effect of moisture. The encapsulated CBT were heated inside the chamber using IR-light source for 3 min at 100, 103, and 105°C to calculate the moisture content. It was hypothesized that the extent of moisture absorption is directly proportional to the amount of hygroscopic surface area on the encapsulated particles.

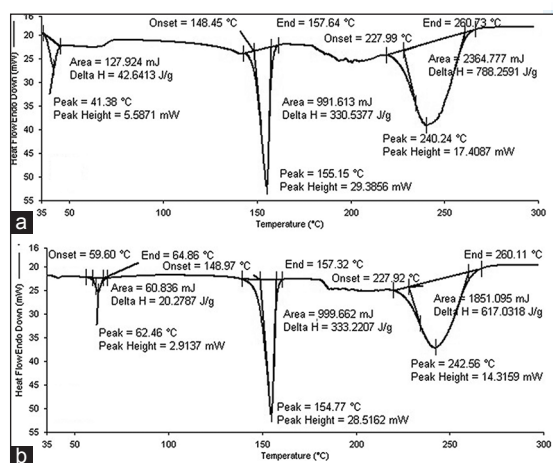


Figure 1: Differential scanning calorimetry thermogram of (a) plain choline bitartrate (CBT) and (b) CBT loaded with formulation excipients

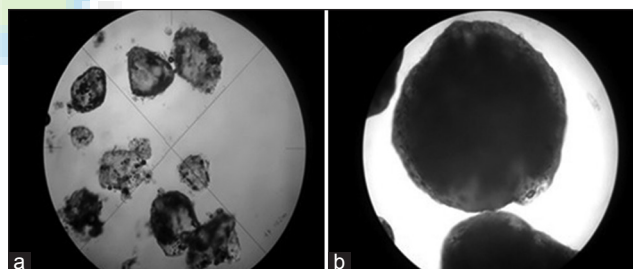


Figure 2: Photomicrograph of (a) plain choline bitartrate (CBT) and (b) microencapsulated CBT

Table 3: Evaluation of pellets for morphological characteristics and flow properties, assay and encapsulation efficiency

Batch	Angle of repose (θ)	Bulk density (g/ml)	Tapped density (g/ml)	Hausner's ratio	Carr's index	Aspect ratio	Assay (%)	Encapsulation efficiency (%)
Plain CBT	39.53±1.24	0.5339	0.7894	1.47±0.21	32.36±1.52	1.86±0.24	99.95±0.98	—
F1	26.32±0.95	0.623	0.8563	1.37±0.19	27.23±1.42	1.92±0.21	90.58±0.71	61.68±1.24
F2	25.21±0.91	0.6532	0.8671	1.32±0.16	24.66±1.36	1.90±0.19	90.65±0.71	61.98±1.21
F3	21.62±0.82	0.7422	0.8514	1.14±0.14	12.82±1.34	1.62±0.17	90.53±0.67	63.61±1.27
F4	20.34±0.85	0.7655	0.8678	1.13±0.12	11.78±1.25	1.23±0.13	90.41±0.62	64.84±1.28
F5	18.92±0.73	0.7500	0.8333	1.11±0.11	9.99±1.19	1.08±0.11	90.30±0.60	65.77±1.29
F6	24.23±0.92	0.6484	0.8734	1.34±0.18	25.76±1.41	1.96±0.27	62.32±0.81	63.65±1.31
1.F7	23.59±0.89	0.6745	0.8845	1.31±0.16	23.74±1.38	1.91±0.23	62.34±0.82	63.76±1.36
F8	21.23±0.87	0.6922	0.8097	1.16±0.15	14.51±1.36	1.54±0.18	62.88±0.71	67.36±1.31
F9	20.12±0.76	0.7012	0.8231	1.17±0.14	14.70±1.31	1.21±0.12	62.24±0.69	68.24±1.32
F10	18.67±0.72	0.6818	0.7894	1.15±0.13	13.63±1.24	1.02±0.10	61.37±0.65	69.37±1.39

CBT: Choline bitartrate

Thus, moisture absorption would be indicative of the intimacy of mixing of HSO with a polymer matrix in encapsulated CBT. The moisture content in encapsulated CBT found to be in the range of $0.73\% \pm 0.23\%$ to $1.1\% \pm 0.24\%$ which is negligible and has no degradation effect on drug efficacy [Table 4]. The drug content analysis was carried out after treatment and observed to be in the range of $90.0\% \pm 1.6\%$ to $90.7\% \pm 1.2\%$ and $60.4\% \pm 1.3\%$ to $61.2\% \pm 1.4\%$ [Table 4] analyzed by potentiometric titration method. No significant change in weight was observed after subjecting the sample to accelerated conditions of temperature and humidity.

Release of encapsulated choline bitartrate

The 5 h release of encapsulated CBT was carried out to determine whether the coating offers significant protection to the choline for its stabilization.^[8] The nonencapsulated CBT gave >60% drug release and thus exhibited discoloration and odor resembling amines. Whereas, the encapsulated CBT 90% and CBT 60% were found to be <20% and 10% drug release depicting less discoloration and odor as shown in Table 5. Therefore, nature of coating offered significant protection when the encapsulate choline exposed to water for 5 h.

Solid state characterization of optimized pellets

Particle size analysis

The volume weighted mean particle size and the d (0.1), d (0.5), and d (0.9) values of optimized pellet formulations and drug (as determined by laser diffractometry) are listed in Table 6. The plain CBT was found to be irregular particle size and shape with higher span value. The optimized pellets of F5 and F10 showed lower span value which expresses their sphericity.^[18]

Differential scanning calorimetry analysis

Differential scanning calorimetry analysis was performed for the plain CBT and optimized pellet formulations to study the drug excipient interactions. The DSC thermogram shows that the crystalline CBT [Figure 4] was characterized by a single, sharp melting endotherm at 155.15°C . DSC thermograms of optimized F5 and F10 batch are also showed in Figure 4. For the formulations F5 and F10, the broad endothermic peaks were observed at 155.72°C and 153.60°C . These endothermic peaks signify that CBT is in a pure state in pellet-based formulations. The results revealed that there was no major interaction or

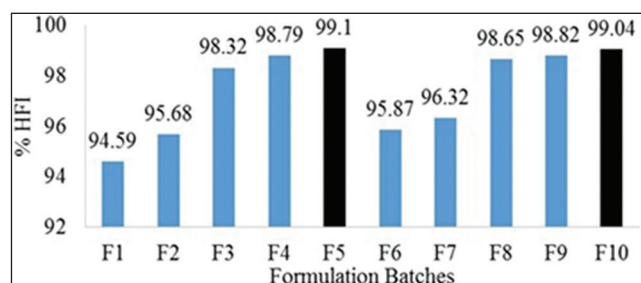


Figure 3: Hardness-friability index of formulations

Table 4: Moisture content and drug content of encapsulated CBT after subjecting to accelerated conditions

Batch	Moisture content (%)	Drug content after accelerated temperature conditions (%)
F1	1.10±0.24	90.67±1.3
F2	0.91±0.24	90.54±1.1
F3	0.88±0.28	90.65±0.98
F4	0.84±0.25	90.54±1.1
F5	0.75±0.23	90.45±1.2
F6	0.95±0.28	62.39±0.93
F7	0.91±0.21	62.54±0.91
F8	0.87±0.27	62.21±0.92
F9	0.89±0.28	62.23±0.87
F10	0.73±0.21	62.10±0.98

CBT: Choline bitartrate

Table 5: Release of encapsulated CBT

Batch	Release of encapsulated CBT (%)
F1	19.68±0.97
F2	19.98±0.92
F3	18.63±0.83
F4	18.84±0.81
F5	17.77±0.74
F6	9.65±0.24
F7	9.76±0.32
F8	8.36±0.21
F9	8.53±0.19
F10	7.33±0.15

CBT: Choline bitartrate

Table 6: Volume weighted mean particle size, the d (0.1), d (0.5), d (0.9), and span values of optimized pellet formulation and plain CBT determined by laser diffractometry

Batch	Mean (µm)	d (0.1) µm	d (0.5) µm	d (0.9) µm	Span value
Plain CBT	326.35	96.288	296.24	603.547	1.712
F5	683.32	396.86	634.46	1046.09	1.023
F10	870.418	649.19	853.62	1111.75	0.542

CBT: Choline bitartrate

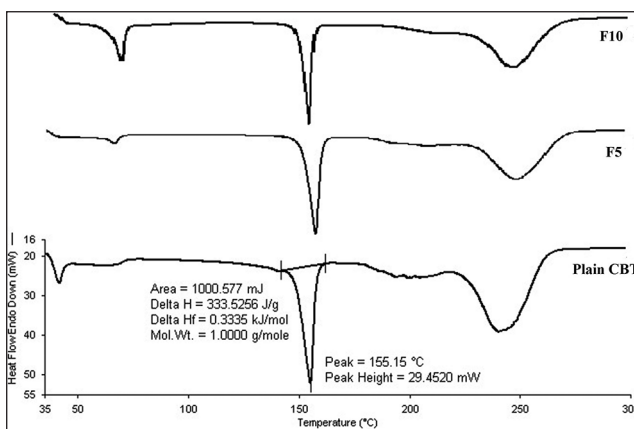


Figure 4: Differential scanning calorimetry thermogram of plain choline bitartrate and optimized F5 and F10

complexation between the drug and the wax throughout the process of pelletization.^[24]

Powder X-ray diffractometry analysis

The powder X-ray diffractograms are shown in Figure 5. It showed sharp multiple peaks, indicating the crystalline nature of the drug. Plain CBT showed sharp peak at diffraction angle (2θ) value of 11.280, 15.120, 16.520, 17.980, 19.060, 21.620, 21.820, 24.00, 26.300, 26.720, and 29.720. These characteristics peaks are indicative for the crystalline form of CBT. The diffractograms of optimized pellets of F5 and F10 showed broad peaks with low intensities, which may attributed to the incorporation of CBT with wax, leading to a slight shift in height of peaks. The PXRD study of pellets revealed retention of crystalline property of CBT in the final product. No significant change in percent crystallinity was observed after coating of wax.^[25]

Fourier transform infrared

Fourier transform infrared spectroscopy was used to assess the interaction between drug-polymer in solid state. In order to evaluate any possible chemical interactions between the drug and carriers, spectra of plain CBT, and optimized pellet formulations were examined. FT-IR spectra of plain CBT showed a stretching vibration at 3322.82 cm^{-1} and 3345.43 cm^{-1} indicating presence of O-H group in tartrate and choline, respectively, C-H stretching at 2918.82 cm^{-1} indicating presence of -CH group [Figure 6]. FT-IR spectra of F5 and F10 showed retention of all the major peaks which indicate the absence of chemical interaction between wax and drug during processing.

Scanning electron microscopy

Scanning electron microscopy study was performed to understand the physical morphology of drug and coated pellet. Plain CBT [Figure 7a and b] was found as crystalline powder with irregular shape and with higher magnification roughness on the surface of the powder was observed. The pellets prepared with HSO [Figure 7c and d] were found as spherical shape pellets, while at higher magnification [Figure 7e and f] showed rough surface with some narrow or round pores on the texture of pellets because of rapid evaporation of water during drying process.^[13] Particle size of optimized pellets was ranging from 900 to 1100 μm .

Stability studies

Stability studies of pellets containing encapsulated F5 exhibited no signs of discoloration or fishy odor development after 4M on storage at accelerated conditions. Furthermore, there was no significant change in the moisture content, and drug content has been observed. But, after 5M slight change in color and fishy odor was developed at accelerated stability studies. The pellets containing encapsulated F10 was exhibited no change in color or fishy odor development after 6M storage [Tables 7 and 8]. It was concluded that developed pellets containing encapsulated F10 were stable for 6 M in accelerated conditions.

CONCLUSION

Microencapsulated pellets of CBT using HSO as encapsulating agent were developed for stabilization of drug by modified extrusion and spheronization technique. The obtained pellets were spherical in shape and relatively smooth surface with a narrow particle size distribution. Extrusion jacketed

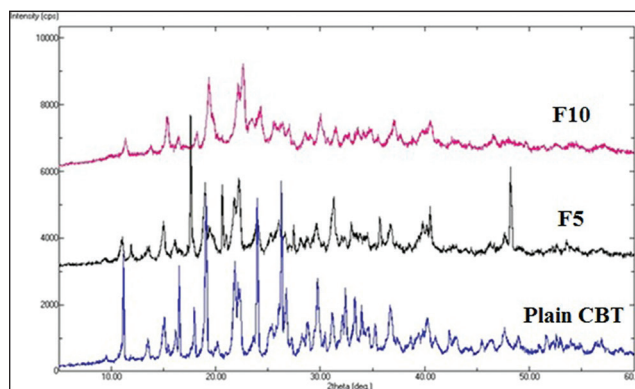


Figure 5: Powder X-ray diffractometry of plain choline bitartrate and optimized F5 and F10

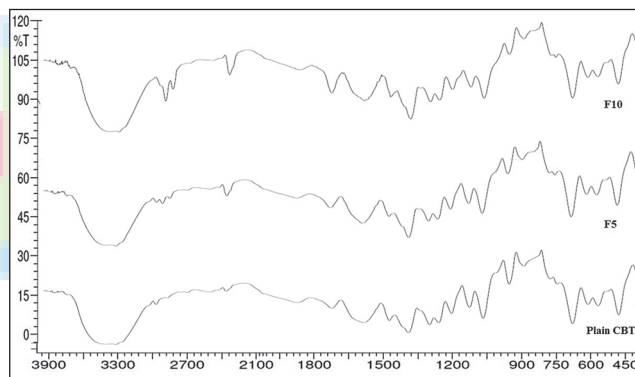


Figure 6: Fourier transform infrared of plain choline bitartrate and optimized F5 and F10

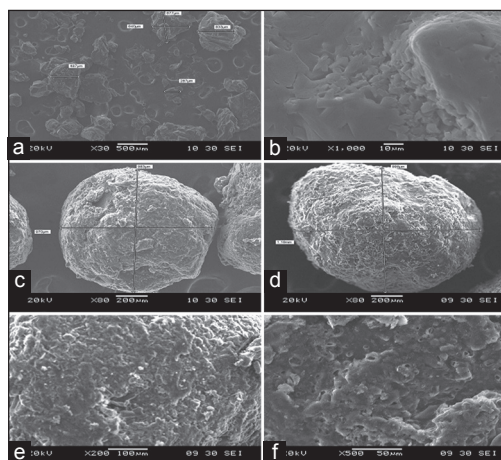


Figure 7: Scanning electron microscopy images of plain choline bitartrate (CBT) (a and b), F5 pellets (c), F10 pellets (d), and CBT pellets surface (e and f)

Table 7: Stability study of encapsulated CBT at 30°C/65% and 40°C/75% (for 1-3 months)

Parameters	Results					
	1-month		2 months		3 months	
	30°C/65%	40°C/75%	30°C/65%	40°C/75%	30°C/65%	40°C/75%
F5						
Moisture content	0.59±0.18	0.60±0.21	0.61±0.20	0.62±0.22	0.64±0.21	0.65±0.23
Assay	90.41±0.32	90.55±0.37	90.62±0.31	90.65±0.38	90.70±0.35	90.75±0.40
Description	White free-flowing	White free-flowing	White free-flowing	White free-flowing	White free-flowing	White free-flowing
F10						
Moisture content	0.59±0.19	0.60±0.21	0.61±0.22	0.62±0.23	0.64±0.24	0.65±0.25
Assay	61.34±0.41	61.55±0.43	61.52±0.44	61.60±0.45	62.30±0.46	62.70±0.48
Description	White free-flowing	White free-flowing	White free-flowing	White free-flowing	White free-flowing	White free-flowing

CBT: Choline bitartrate

Table 8: Stability study of encapsulated CBT at 30°C/65% and 40°C/75% (for 4-6 months)

Parameters	Results					
	4 months		5 months		6 months	
	30°C/65%	40°C/75%	30°C/65%	40°C/75%	30°C/65%	40°C/75%
F5						
Moisture content	0.67±0.24	0.70±0.26	0.73±0.28	0.77±0.32	0.74±0.31	0.80±0.35
Assay	90.74±0.38	90.80±0.42	90.79±0.41	90.84±0.45	90.82±0.43	90.86±0.47
Description	White free-flowing	White free-flowing	White free-flowing	Slight change in color and Fishy odor	Slight change in color and Fishy odor	Yellow spots on granules and Fishy odor
F10						
Moisture content	0.67±0.26	0.70±0.28	0.73±0.29	0.77±0.30	0.74±0.31	0.80±0.33
Assay	62.80±0.48	62.85±0.51	62.82±0.51	62.87±0.52	62.89±0.53	62.91±0.55
Description	White free-flowing	White free-flowing	White free-flowing	White free-flowing	White free-flowing	White free-flowing

spheronization technique presents a faster and cheaper alternative compared to conventional coating methods whereas solvent evaporation and spray drying become very expensive and time-consuming. Optimized both the formulation, CBT 90% (F5) and CBT 60% (F10) were found to be stable for 4M and 6M, respectively, at accelerated conditions.

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