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Research paper

Microencapsulation of lutein extracted from marigold flowers (*Tagetes erecta* L.) using full factorial design

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ABSTRACT

Lutein (LUT), is one of the most important carotenoids having prominent antioxidant activity. However, its use is limited due to poor solubility and instability under adverse conditions. LUT was microencapsulated with soluble polymers using spray drying to improve its solubility and bioavailability. Maltodextrin (polysaccharide base) and copovidone (polyvinyl pyrrolidone vinyl acetate based copolymer) were evaluated as hydrophilic carriers for encapsulation of LUT. Design of Experiments (DOE) was utilized and microencapsulation process was optimized using full factorial design. Copovidone proved to be better carrier compared to maltodextrin and showed enhanced dissolution and antioxidant characteristics of LUT. Microencapsulated LUT powder was characterized by dissolution study, DSC, XRD and SEM. This study can be used as a guideline for optimization of microencapsulation of similar bio-actives with hydrophilic carriers to improve solubility and subsequent bioavailability.

1. Introduction

Lutein (LUT) is one of the most significant carotenoids present in the humans obtained as a pigment from plants. Humans are unable to synthesize carotenoids *de novo* and, as a result, its presence in human tissues is exclusively of dietary origin. Lutein along with zeaxanthin are known as macular pigments; derive this name due to the fact that they are found in higher amounts in the macula (central portion of the retina) [1]. LUT, which is devoid of pro-vitamin A activity in humans, exhibits various other biologically significant activities; notably the anti-proliferative activity. It has thus been proposed as nutritional supplement in special cases [2]. Carotenoids, which are antioxidants are found abundantly in fruits and plants and hence could be of value in diseases aggravated by stress and free radicals. LUT has very poor solubility in water and hence its bioavailability is also extremely low. Many researchers have worked to improve solubility and bioavailability using various approaches such as SNEDDS technology [3–5], liposomal delivery [6–8], nanoemulsions [9,10], nanocapsules [11], encapsulation with polysaccharides [12,13] and encapsulation with hydrophilic polymers [14–16].

Microencapsulation is widely used, well known technology for improving stability of oils and fatty acids against oxidation. It is used for masking undesirable odours and flavours in dry powder form finished products. Microencapsulation can be defined as a process in which tiny droplets, namely core, are surrounded by a coating of a microencapsulating agent. This process converts oily or waxy active materials into free flowing powder which can be easy to use in pharmaceuticals, nutraceuticals and food industries. The coating wall materials can be made up of variety of food grade excipients such as Polysaccharides, Celluloses or Polymers which provide a physical barrier against environmental conditions. The simplest of the microcapsules or microspheres (size range from 1 μm to 800 μm) may consist of an active core surrounded by a wall of coating material of uniform or non-uniform thickness. The core may be composed of one or several ingredients and the wall may be single or double-layered [17]. Among all the microencapsulation techniques, many polymeric excipients like polysaccharides, celluloses, povidone are considered to be good encapsulating agent because of their good solubility and encapsulation efficiency [18,19]. Maltodextrin, gelatine and porous starch have been specifically used for microencapsulation of LUT for improving its stability [20,21].

Design of Experiments (DOE) is a tool which allows formulators to understand the effects of formulation variables on desired performance outputs of the product. Using DOE, the relationship

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between different independent variables can be established and final product performance quality with respect to critical quality attributes (CQAs) can be determined. DOE was successfully used in pre-formulation and formulation studies to facilitate the screening process of excipients, to study the effects of different formulation and/or process variables or to determine the best levels of excipients that provide optimal levels of CQAs [22–25]. In recent years, the pharmaceutical industry has used experimental design more for the optimization of pharmaceutical agents; however, only a few are reported in the literature for the development of dosage forms [26].

In this study LUT microencapsulation formulations were optimized for desired CQAs like wetting time, dissolution rate and antioxidant inhibition activity by using full factorial design approach. Two different types of carrier bases, maltodextrin (polysaccharide base) and copovidone (polyvinyl pyrrolidone polyvinyl acetate copolymer, PVA/VA base) were compared for encapsulation efficiency. The approach enables simultaneous evaluation of the effects of different variables such as type of carrier/wall material, ratio of carrier, concentration of solubilizer and homogenization speed and possible interaction within these factors. This selected Quality by Design (QbD) approach was used for screening the best compositions and best suited experimental conditions within short period of time and with minimum trial runs.

2. Materials and methods

2.1. Materials

Lutein (LUT) is a xanthophyll concentrate extracted from petals of marigold flowers *Tagetes erecta* L. LUT was procured from OmniActive Health Technologies (India); povidone K30 (Kollidon® K30) and copovidone (Kollidon® VA64) were obtained as gratis samples from BASF (India); maltodextrin (Glusidex IT19, DE value 17–19) was obtained as gift sample from Roquette Feres (France); Tween 80 was gifted by Croda Ltd. (India). All the other excipients and chemicals were of pharmaceutical or analytical grade and were used as received.

2.2. Preparation of encapsulated LUT

In preliminary trials maltodextrin, povidone K30 and copovidone were used individually as carrier system for encapsulation of LUT. The different formulations studied are as shown in Table 1. Natural tocopherol at a concentration of 4.0% w/w and 0.50% w/w of colloidal silicon dioxide were added to this mixture. Natural tocopherol was used as a preferred antioxidant for stabilization of LUT and colloidal silicon dioxide as anti-sticking agent to avoid agglomeration of the spray dried micro particles. LUT was dissolved in appropriate quantity of dichloromethane while hydrophilic carrier was uniformly dispersed in appropriate quantity of isopropyl alcohol (2- propanol) under stirring as per Table 1. LUT solution was added into carrier polymer dispersion slowly upto 30 min with constant stirring at 500 rpm. Resulting dispersion was homogenized at a speed of 8000 rpm using lab homogenizer IKA T25 (Ultra Turrax, Germany) for 2 h. The resulting LUT dispersion with hydrophilic carrier was filtered through sieve of 200 μm size. This solution was kept under stirring and spray dried using table top spray dryer (model LU-227, Lab-Ultima, India) having concurrent flow arrangement with expansion chamber height of 45.0 cm and internal diameter of 15.0 cm. The spray nozzle used was of 1.0 mm size. The operating conditions maintained were inlet temperature 100–120 °C, outlet temperature 60–65 °C, flow rate of the solution 300 mL/h, airflow rate 40–50 m^3/h and atomizing air pressure 1.2–1.5 bar. The spray dried micronized powder was collected from the cyclones and passed through US ASTM mesh of size 40. The spray dried powder was further dried under vacuum for 12 h at temperature of 45 ± 2 °C to remove the traces of isopropyl alcohol and dichloromethane. The total residual solvents present in powder samples was checked by using Gas chromatography (PerkinElmer, Clarus-480, USA). All the samples showed total residual solvents below detectable limits. This powder was kept in thermally sealed aluminium pouch under vacuum until further evaluation [27].

As per the full factorial design identified critical parameters, type of carrier used was maltodextrin and copovidone. The ratio of carrier to LUT was kept 2:1 to 4:1 on dry w/w basis. Tween 80 (non-ionic surfactant) was used as a solubilizer in concentration range of 2% w/w to 6% w/w. The homogenization speed was varied from 8000 to 12000 rpm. All other spray drying and formulation parameters were kept same for all the DOE run orders.

2.3. Encapsulation efficiency

Encapsulation efficiency was calculated by analyzing the LUT content in the microencapsulated spray dried powder. UV visible spectrometry (UV-1601 Shimadzu, Japan) was used to quantify the LUT content in microencapsulated spray dried powder at λ_{max} 446 nm. LUT was extracted by solubilising microencapsulated spray dried powder in 5 mL aliquots of dichloromethane. The solution was vortexed for 2 min and further centrifuged at 1500 rpm for 15 min for separating insoluble residue and organic layer. The organic layer was collected by repeating the process twice. The collected dichloromethane layer was evaporated under nitrogen till

Table 1
Preliminary spray drying trials and results of LUT formulations.

Ingredients	Formulations of LUT[a]							
	LUT	A1	A2	A3	A4	A5	A6	A7
LUT	31.8	31.8	31.8	31.8	31.2	31.2	23.3	23.3
Povidone K 30	–	63.7	0.0	0.0	0.0	0.0	0.0	0.0
Copovidone	–	0.0	63.7	0.0	62.3	0.0	70.2	0.0
Maltodextrin	–	0.0	0.0	63.7	0.0	62.3	0.0	70.2
Tween 80	–	0.0	0.0	0.0	2.0	2.0	2.0	2.0
Natural Tocopherol	–	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Colloidal silicon dioxide	–	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Dichloromethane	–	454.0	341.0	454.0	341.0	332.0	332.0	332.0
Isopropyl Alcohol	–	318.0	431.0	318.0	431.0	440.0	440.0	440.0
EE (%)	–	96.3 ± 1.2	95.2 ± 0.7	96.5 ± 1.1	96.2 ± 1.3	96.2 ± 0.6	96.7 ± 1.2	95.7 ± 0.7
DR30 (%)	17.8 ± 0.4	24.0 ± 0.2	32.2 ± 0.3	35.2 ± 1.3	42.3 ± 0.3	45.0 ± 0.2	51.4 ± 1.7	55.6 ± 3.1
IC50 (μg/mL)	48.0 ± 2.3	41.0 ± 1.4	40.2 ± 2.0	38.3 ± 2.3	38.0 ± 2.3	37.0 ± 1.4	30.0 ± 1.3	28.5 ± 1.6

[a] All values are in gram for 100.0 g batch size.