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D-optimal Mixture Design for Optimized Microencapsulation of Vitamin A Palmitate and Its Characterizations

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Abstract: Food fortification is an effective intervention strategy for combating micronutrient deficiencies. Vitamin A can be fortified in a wide range of food vehicles. In this research work, vitamin A in the form of palmitate (VAP) have been microencapsulated by emulsion technology intended for fortification purposes. D-optimal mixture design approach was applied for optimizing the experimental process parameters. Detailed physico-chemical –thermal characterization of the optimized VAP emulsion was done. VAP content was kept constant as per fortification limit. Desirability ramp function graphs and lab experimentations showed maltodextrin (2.5-2.7), OSA starch (1.5-1.6), Tween 80 (1.5-1.7), Span 40 (2.0-2.2) in terms of percentages gave encapsulation efficiency in the range of 98.2-98.8. The optimized VAP emulsion showed particle size in the range of 940-942 nm, polydispersity index 0.41, zeta potential (ζ) value ranging from -31.25 to -32.01 mv; viscosity and interfacial tension was determined to be 1.561±0.03 mPaS and 22.6 mNm−1 respectively. Low temperature DSC studies (-5ºC to 90ºC) of VAP emulsion showed sharp endothermic peaks of vitamin A that disappeared with further elevation in temperature and some flat thermograms of other excipients. The vitamin A content in VAP emulsion was determined to be 88.45±0.03%, the content being reduced to 86.01±0.04% after storage under accelerated conditions (75ºC, 80% RH). The formulated VAP emulsion showed high encapsulation efficiency and significant stability of the emulsion system.
Keywords: Food fortification; Vitamin A; Microencapsulated; Emulsion technology; D-optimal mixture design; Encapsulation efficiency; Stability.

1. Introduction

Vitamins are organic compounds essential for growth and development. Mostly they are not synthesized by animals and humans and are to be supplemented through diet. Vitamin A, also including the carotenoids and retinoids are fat soluble, polyunsaturated hydrocarbons whose importance for vision health has been recognized in the early 1500 BC in ancient Egypt. Beyond the crucial role of vitamin A in vision, the essential role of this vitamin (especially the retinoic acid components) in immune function, reproduction and embryonic development, cellular growth and differentiation, maintaining the integrity of epithelial cells, its important role in childhood development and nourishment, ocular health, and strengthening the immune system. Vitamin A deficiency effects have been estimated globally either at clinical or sub clinical level. Since, vitamin A can't be synthesized the de novo it must be provided through diet [1-5]. Indian Council of Medical Research (ICMR) has set recommended dietary allowance (RDA) of 900 micrograms and 700 micrograms daily for men and women respectively which are also 3000 IU (international unit) and 2300 IU respectively. Here it is to be mentioned that an international unit is an internationally accepted amount of a substance whose value depends on the biological activity or effect of the substance. Such units are used for the fat-soluble vitamins (such as vitamins A, D and E) and certain hormones, enzymes, and biologicals (such as vaccines). Considering the case of children, those aged between 1 to 8 years require 400-600 µg/day and those children above 8 years require 600-800 µg/day of vitamin A [3, 5]. To combat vitamin A deficiency (VAD), food fortification with vitamin A is a successful intervention strategy. This vitamin being fat soluble, edible oils are important fortifying vehicles for vitamin A as the fortificant. Dried milk and flavored milk powders are also being fortified with vitamin A. Milled wheat flour is also a suitable candidate vehicle for vitamin A fortification. Other successful staple food vehicles for vitamin A fortification as reported in different public health programs include dairy products like fats and margarines, raw or refined sugar, maize four, corn meal, and other condiments and seasonings [1, 6, 7]. However due to high degree of unsaturation in the molecule vitamin A is highly labile under the ambient conditions. Photocatalytic degradation of retinoids is accelerated in presence of oxygen. Vitamin A being hydrophobic rapidly degrade in aqueous media. Different esterified forms of vitamin A like palmitate also rapidly degrade in aqueous solutions due to lower polarity and

thus poor aqueous solubility of retinoids [1, 3]. Microencapsulation is a suitable protective solution against its chemical instability during storage, and also enhances the dispersibility of the vitamin A esters with expected improved bioavailability [5].

Different methods of microencapsulation of vitamin A have been reported in the literature. Nano or micro emulsification followed by spray drying of the resultant emulsion gives rise to vitamin loaded powdered microcapsules. Different oil-in-water emulsions have been developed with various emulsifying and stabilizing agents for the encapsulation of vitamin A. Emulsification techniques are also opted for developing lipid based nanocarrier (NC) systems for dermal applications of vitamin A. Lipospheric vesicles of solid lipid nanoparticles (SLNs) loaded with retinol or vitamin A are introduced into oil-in-water emulsion systems for the successful delivery of the vitamin. However the novel formulations of SLNs, NC are mostly suitable for the dermal application of vitamin A. Other retinyl palmitate loaded polymeric nanoparticles are reported for the treatment of dermal disorders. Biodegradable microspheres loaded with vitamin A are also prepared [2, 5, 8-15].

Emulsion systems have become an integral part of food formulation and an effective delivery vehicle especially for lipophilic components like vitamin A. Thus, this research article reports the microencapsulation of vitamin A palmitate (VAP) by emulsification method. D-optimal mixture design approach was applied for optimizing the experimental process parameters and quality control studies (detailed physico-chemical and thermal characterization) of the encapsulated VAP.

2. Materials and methods

Analytical grade chemicals like vitamin A palmitate, vitamin E, Tween 80, cremophore RH, maltodextrin, OSA modified starch were purchased from companies Sigma, India and Merck, India. Some equipments used for the experimentations include: Electronic balance (Digital Analytical balance), UV spectrophotometer (Analytical technologies limited, TS2090), pH meter (Chemiline), centrifuge (Remi, R-8C Lab Centrifuge), stirrer (Tarsons), FTIR spectrometer (Thermo Fisher). Design expert software (version 7.0) was used for optimization of experimental process parameters.

2.1 Compatibility studies of VAP with other encapsulation components by FTIR analysis

Fourier Transform Infrared (FTIR) spectroscopy was applied in order to study the compatibility amongst the fortificant i.e. vitamin A (in the form of vitamin A palmitate or VAP) along with vitamin E (added antioxidant) and the excipients (e.g. surfactants, emulsifiers, food additives and thickeners) used for the purpose of encapsulation by emulsification technology. An initial trial sample of VAP emulsion was prepared whose FTIR spectra was recorded along with the individual FTIR spectra of the excipients used. Comparison was done to see if there are any drastic changes in the spectra due to interactions amongst the excipients and the fortificant i.e. vitamin A palmitate (VAP). FTIR spectra were recorded in FTIR spectrometer (Thermo Fisher) and were analyzed using OMNIC software.

2.2 Chemometrics optimized experimental process parameters for microencapsulation of VAP by emulsification

An empirical modeling technique for finding out the relationship amongst set of experimental factors and observed results is Response Surface methodology (RSM). The sequential steps in RSM involves developing the polynomial equation, generation of statistically analyzed analysis of variance (ANOVA) table, generation of response surface plots with different combinations of input parameters, determining the optimal level from desirability analysis and confirmation test for validation [16-19].

D-optimal mixture design approach using Design Expert software (version 7.0) was employed for optimizing the experimental process parameters for emulsification of VAP. Since VAP was encapsulated for the purpose of fortification, the amount of the fortificant (VAP) was calculated as per ICMR fortification limits and was thus kept constant. The percentages of Tween 80 (1.5-2.5), cremophore RH (2-4), maltodextrin (2.2-6.6) and OSA (1.5-3.5) are the input variables. Polydispersity index or PDI, encapsulation efficiency or EE, and particle size are the output variables. The trial runs provided in the software generated experimental layout was followed to carry out the lab experimentations [16-20]. The parameters PDI, EE and particle size were considered as the output variables. The output variables were determined for each trial run. These experimental values were incorporated in the datasheet of the experimental layout generated by the software and the data were statistically analyzed by means of analysis of variance or ANOVA followed by generated models optimization by Design Expert generated desirability ramp function graphs [16-20]. During selection of the best optimized model, amongst three output variables EE or encapsulation efficiency was considered for optimizing the process parameters for the devised microencapsulation procedure for the fortificant (VAP). The devised procedure gave a maximum EE of 98%.

As a outcome for the optimization of experimental process parameters with the aid of Design Expert software with subsequent lab experimentations, software generated tables were obtained on model adequacy, fit summary

statistics of the devised model, statistical analysis for each of the out variables by ANOVA, and the software generated graphs include normal plot of residuals for studentized diagnostics, 3D surface plots the model graphs showing the experimental process parameters and the desirability ramp function graphs basing on which final process optimizations were done [16-20].

2.3 Fixing the fortifiable amount of VAP

Vitamin A palmitate (VAP) is an ester of retinol and palmitic acid which is available in oily or dry forms. For the purpose of vitamin A fortification firstly ICMR recommended admissible fortification limit of 2000 IU/qt (IU-international unit, qt-quart) was followed. Here 1 quart is equal to 0.946L and equivalent to 0.25 gallons.

2.4 Methodology for encapsulation of VAP

VAP was encapsulated by emulsification technology. The lipid phase was prepared by mixing VAP and vitamin E (as antioxidant in 1IU~0.67mg) with cremophore RH 40 (non ionic solubilizer and emulsifying agent), heated to 60°-65°C along with the dropwise addition of Tween 80 (non ionic surfactant and emulsifier). The aqueous phase was prepared by dissolving maltodextrin (water soluble plant based polysaccharide used as food additive and thickener) with OSA modified starch (octenyl succinic anhydride modified starch, facilitates emulsification with thickening effect) and caseinate (food emulsifier and thickener), heated at 50°C and was subjected to magnetic stirring for about 1.5h at 900 rpm. Next the lipid phase was added drop wise to the aqueous phase with high speed homogenization (1200 rpm) for 2 hr. The detail of the work methodology is given in the form of a flowchart (Fig 1).

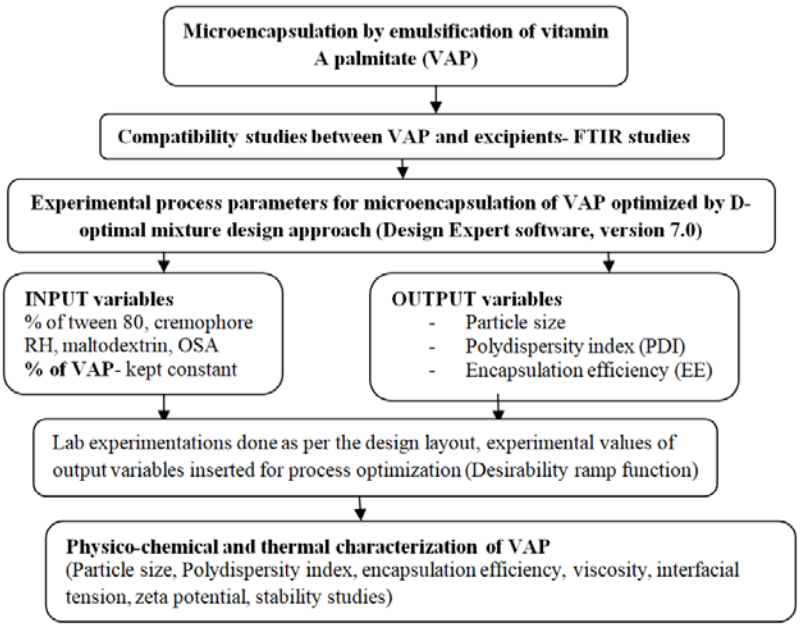

Fig 1. Flowchart of detailed work methodology

2.5 Physico-chemical and thermal characterization of VAP microemulsions

Vitamin A palmitate (VAP) was encapsulated by emulsion technology. The fortificant VAP got dispersed between the aqueous and lipid phase in the form of droplets; the dispersed droplets of the fortificant got coated with the layers of incorporated additives. Particle size, polydispersity index (PDI), viscosity and interfacial tension, Zeta potential, encapsulation efficiency, fortificant or vitamin A content, and stability studies are the essential physico-chemical parameters of the VAP emulsion were studied.

2.5.1 Particle size and polydispersity index

Images of A few drops of VAP emulsion were smeared in a clean grease free slide and covered with a cover slip were taken using a Leica DM2500 LED microscope coupled with Leica DFC7000T camera and a LAS X software.

Determination of zeta potential (ζ) was done by electrophoretic mobility where the sign and magnitude of the particle charge was determined by the instrument by measuring the direction and velocity of the movement after placing the sample solution in capillary tubes between the electrodes [20, 21].

2.5.2 Viscosity and interfacial tension

Bohlin CVO rheometer (Malvern Instrument, Malvern, UK) with a cone and plate geometry (CP 4ºC/ 40 mm diameter) at a gap of 150 µm was used for determining the shear viscosity of the VAP emulsion samples. Prior to experimentation, the instrument was equilibrated at 25ºC after loading the sample in rheometer measurement plate for 5 min. At shear rates of 0.01 to 100s-1 , shear viscosity measurements were carried out. Newtonian motion was observed for the two different phases of VAP emulsion (the aqueous and the organic phase). The shear measurements were carried out at a fixed shear rate (10 s-1) [20].

Drop shape analysis method was followed for measuring interfacial tension at the oil- water interface. Following the mentioned methodology the aqueous and oily phase (organic) of the VAP emulsion was prepared separately. Before measuring interfacial tension by drop shape analysis method, under equilibrium conditions the oily phase was injected into the aqueous phase and then interfacial tension was measured [20, 22].

2.5.3 Encapsulation efficiency

The *encapsulation efficiency* (%) of the devised method was calculated as per the equation [21] provided below:

$$\text{encapsulation efficiency}(\%) = \frac{\text{Total vitamin } content\ (\text{g}) - \text{ vitamin on surface (g)}}{total\ vitamin\ content\ (g)} X100$$

The above equation was followed for determining the *encapsulation efficiency* of the formulated VAP emulsion. From the sample of the VAP emulsion where vitamin A is in encapsulated state, was extracted by adding about 5mL of hexane followed by 30s of vortexing and then centrifugation for about 5 min at 4000 rpm. 1 mL aliquot of the supernatant was withdrawn for determining the vitamin A content at the surface spectrophotometrically after interacting the aliquot containing vitamin A with 50% trichloroacetic acid solution in dichloromethane to get a blue reaction product detected at 620 nm [22].

2.5.4 Vitamin A content

The VAP emulsion was diluted with isopropanol and the absorbance of the solution was recorded in spectrophotometer at 325 nm with isopropanol as the solvent. Vitamin A content was calculated by comparing the measured absorbance values with those in the standard curves [22, 23].

2.5.5 Stability studies

The main purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light, enabling recommended storage conditions, re-test periods and shelf-lives. The *stability* of the VAP emulsion was initially checked visually by keeping the emulsion samples in measuring cylinders undisturbed. The samples were checked for creaming, phase separation etc. The stability was measured by % of separation given by the equation:

$$\%\ separation = \frac{H1}{\text{H0X}}$$

here H_0 is the initial height of the emulsion and H_1 is the upper phase height.

Observing the rate of product degradation at room temperature is time consuming. To avoid such undesirable delay, *accelerated stability studies* have been adopted. The optimized batch of VAP emulsion was selected for stability studies. Some of the test samples were stored at (55ºC, 75% RH) and some at (75ºC, 80% RH) in closed high density polyethylene bottles for 7 days. The samples were withdrawn after 7 days and were analyzed for vitamin A content spectrophotometrically [20, 22, 23].

Low temperature differential scanning calorimetric studies (LT-DSC, Perkin Elmer Pyris Diamond DSC) of VAP emulsion was carried in the temperature range of -5ºC to 90ºC.

3. Results and discussion

3.1 FTIR analysis for compatibility studies

A compatibility study was done by FTIR analysis. The individual FTIR spectra of vitamin A palmitate (VAP) and the excipients were overlapped (Figure 2) to clearly show that there were no significant alterations in the overlapped spectra obtained after intermixing them. Compatibility amongst the fortificant and the excipients is an important pre requisite while developing an encapsulation process. Each components of the VAP emulsion i.e. fortificant vitamin A as well as the excipients used showed their own distinctive structural features. Obviously due

to chemical compatibility between the fortificant (vitamin A) and chosen excipients there were no chemical interactions between them.

Interpretation of FTIR spectra of the components of the VAP emulsion are discussed below:

The peaks in FTIR spectra of *vitamin A (the fortificant)* are mostly due to the functional groups (methyl, ester, hydroxyl) of vitamin A. Peaks due to stretching of the CH_3 groups were observed at 2853 cm^{-1} and 2922 cm^{-1}, O-C=O stretching showed bands at 1739 cm^{-1}, aromatic cyclohexene stretching showed peaks at ~1500 cm^{-1}, the peaks within 1460 cm^{-1} to 1160 cm^{-1} are due to –CO group stretching.

In FTIR spectra of *Vitamin E (the antioxidant in VAP emulsion)*, the terminal –OH group showed a broad band at 3473 cm^{-1}, -CH_2- showed band at 2923 cm^{-1} due to asymmetric stretching and –CH_3- showed band at 2864 cm^{-1} due to symmetric stretching, skeletal phenyl showed band at 1457 cm^{-1} and the peaks at 1377 cm^{-1} (due to methyl symmetric bending), 1262 cm^{-1} (-CH_2 groups), 1080 cm^{-1} and 919 cm^{-1} (plane bending of phenyl and trans =CH_2 and C-O stretching) are observed in the FTIR spectra of vitamin E that are mostly the hydroxyl, methyl, ester, phenyl groups in vitamin E.

FTIR spectra of Tween 80 showed strong broad absorption band at 3498 cm^{-1} and 3436 cm^{-1}due to –OH stretching, CH_3 groups showed bands at ~2900 cm^{-1}, -CH_2 group showed asymmetric and symmetric stretching at 2860 cm^{-1}, C=O ester group and C-O-C stretching showed absorption bands at 1734 cm^{-1} and 1094 cm^{-1}.

The *FTIR spectra of OSA modified starch* showed some new peaks in comparison to normal starch. The new peak at 1571 cm^{-1} is due to the asymmetric stretching vibration of the carboxlate RCOO-, another peak at 1725 cm^{-1} is attributed to the C=O stretching vibration of the ester carbonyl group.

The *FTIR spectra of maltodextrin* showed absorption band at 3399 cm^{-1} due to OH group, the characteristic band for polysaccharide was observed at 980-1200 cm^{-1} and the carbonyl group showed stretching band at 1152 cm^{-1} and 1018 cm^{-1}.

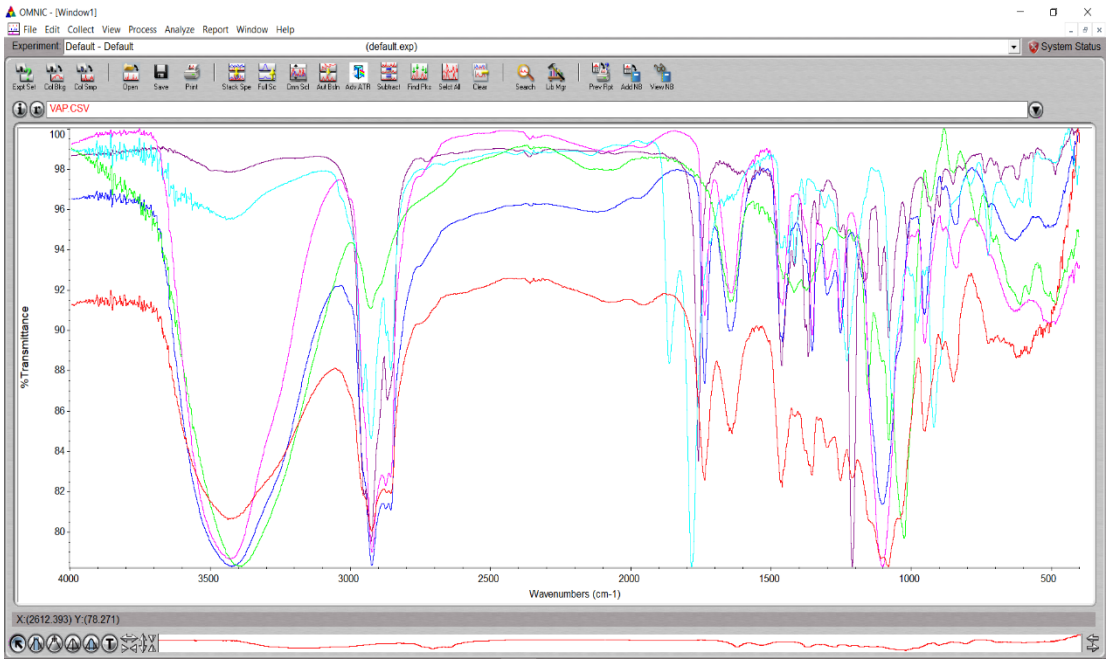

Fig 2. Overlapped FTIR spectra (vitamin A -red, vitamin E-blue, maltodextrin-violet, OSA modified starch-light green, tween 80- light blue, VAP emulsion-pink). No significant changes in the overlapped spectra suggests no interactions amongst fortificant and the excipients used

3.2 Design expert assisted optimization of encapsulation process parameters

The model graphs in the form of 3D surface plots (Figure 3) generated by the Design expert software considering particle size, polydispersity index (PDI) and encapsulation efficiency (EE) as the responses helped to evaluate and interpret the results.

The normal probability plot in the form of Normal plot of residuals indicates whether or not the residuals follow a normal distribution. Here less scattering of points following a straight line is expected. In the method for optimizing encapsulation process parameters of vitamin A, with three outputs (particle size, PDI, EE) as a response the normal plot of residuals showed very less scattering justifying the suitability of the method. The three ANOVA tables (Table 1-3) for statistical analysis of the developed encapsulation method for vitamin A generated by software justified the significance of the model in each case.

From the software generated ANOVA tables (Table 1-3), the model for the three responses i.e. particle size

(Table 1), PDI (Table 2), EE (Table 3) were significant with p-values of <0.0001. As analyzed and generated by design expert software, the Model F-values of 63660000.0 for response 1 (particle size), 28315432.00 for response 2 (PDI), and 257226.86 (EE) for response 3 implied that the model as well as the model terms are significant. The final step is the numerical optimization of the statistically analyzed model with the help of desirability ramp function graphs. Method optimization aims to maximize, minimize or obtain the target value of the response. In chemometrics, desirability is an objective function that ranges from zero outside of the limits to one at the goal [20]. The desirability ramp function graphs (Figure 4) showed the optimized experimental process parameters for encapsulation of vitamin A with 98% encapsulation efficiency (results of lab experimentation as carried out by software generated experimental layout).

Amongst the output responses major focus was given on EE. Considering the percent of input parameters, maltodextrin (2.5-2.7), OSA starch (1.5-1.6), Tween 80 (1.5-1.7), Span 40 (2.0-2.2) gave encapsulation efficiency in the range of 98.2-98.8 with particle size in the range of 940-942 nm, as observed in desirability ramp function graphs. However as per lab experimentation results, maltodextrin (2.6%), OSA starch (1.5%), Tween 80 (1.6%), Span 40 (2.1%) showed 98.8% encapsulation efficiency and is considered as the optimized methodology.

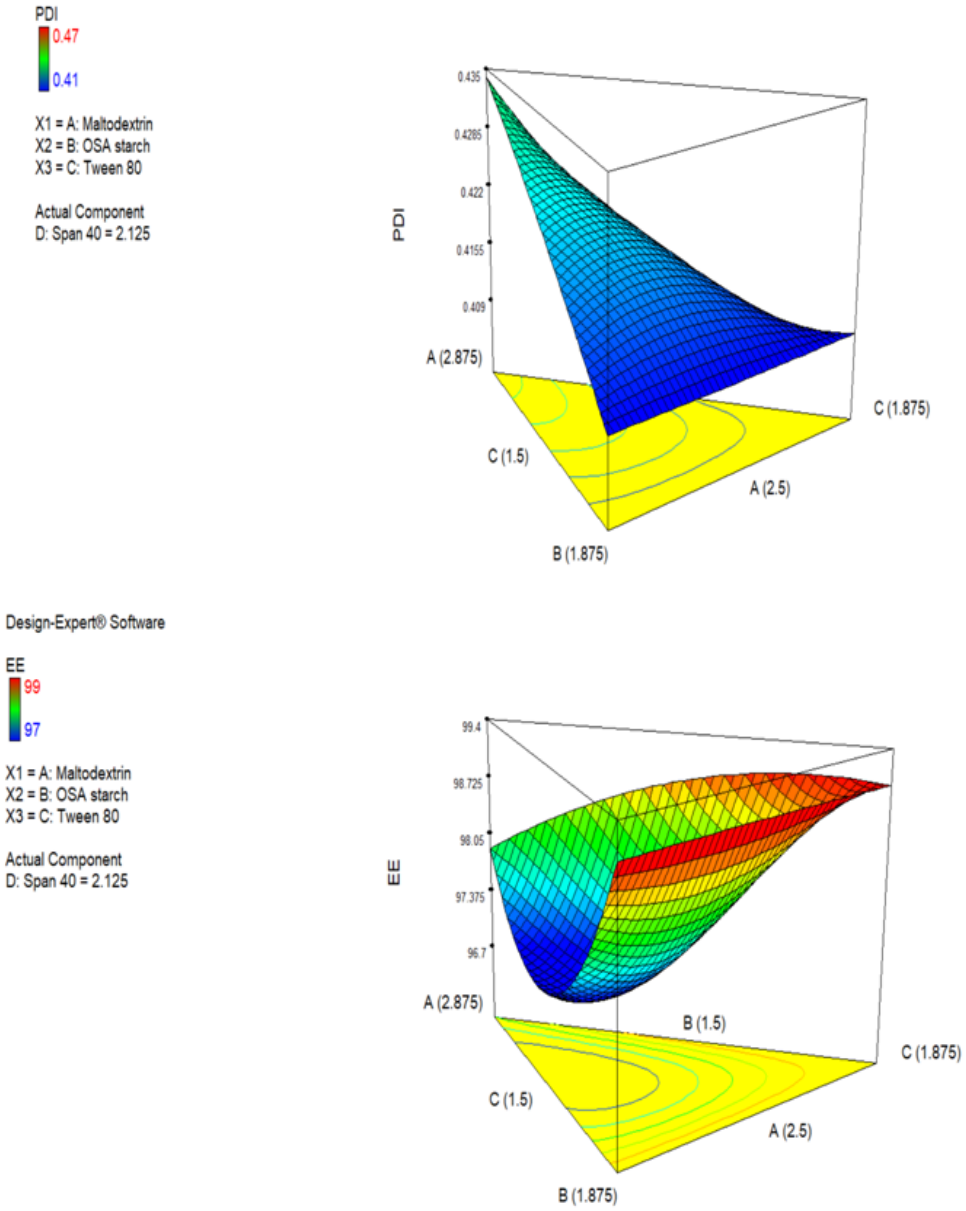

Fig. 3. Model graphs as 3D-surface plots generated by Design expert software for optimization of process parameters for vitamin A encapsulation

Table 1. Particle size (Response 1) for vitamin A encapsulation analyzed by ANOVA.

Source	Sum of squares	df	Mean square	F value	p-value Prob>F	
Model	212.20	13	16.32	6.366E+007	<0.0001	*Significant*
Linear mixture	204.48	3	68.16	6.366E+007	<0.0001	
AB	3.36	1	3.36	6.366E+007	<0.0001	
AC	0.62	1	0.62	6.366E+007	<0.0001	
AD	0.17	1	0.17	6.366E+007	<0.0001	
BC	1.53	1	1.53	6.366E+007	<0.0001	
BD	0.77	1	0.77	6.366E+007	<0.0001	
CD	0.022	1	0.022	6.366E+007	<0.0001	
ABC	1.58	1	1.58	6.366E+007	<0.0001	
ABD	1.11	1	1.11	6.366E+007	<0.0001	
ACD	0.025	1	0.025	6.366E+007	<0.0001	
BCD	1.889E-004	1	1.889E-004	6.366E+007	<0.0001	
Residual	0.000	6	0.000			
Lack of fit	0.000	1	0.000			
Pure error	0.000	5	0.000			
Cor Total	212.20	19				

Table 2. Polydispersity index or PDI (response 2) for encapsulation of vitamin A analyzed by ANOVA.

Source	Sum of squares	df	Mean square	F value	p-value Prob>F	
Model	6.580E-003	13	5.062E-004	2.832E+007	< 0.0001	*Significant*
Linear mixture	5.708E-003	3	1.903E-003	1.064E+008	< 0.0001	
AB	1.141E-004	1	1.141E-004	6.383E+006	< 0.0001	
AC	7.400E-005	1	7.400E-005	4.140E+006	< 0.0001	
AD	5.102E-005	1	5.102E-005	2.854E+006	< 0.0001	
BC	2.995E-005	1	2.995E-005	1.675E+006	< 0.0001	
BD	4.308E-005	1	4.308E-005	2.410E+006	< 0.0001	
CD	1.116E-005	1	1.116E-005	6.241E+005	< 0.0001	
ABC	3.106E-005	1	3.106E-005	1.738E+006	< 0.0001	
ABD	6.170E-005	1	6.170E-005	3.452E+006	< 0.0001	
ACD	1.369E-005	1	1.369E-005	7.661E+005	< 0.0001	
BCD	4.507E-009	1	4.507E-009	252.15	< 0.0001	
Residual	1.073E-010	6	1.788E-011			
Lack of fit	1.073E-010	1	1.073E-010			
Pure error	0.000	5	0.000			
Cor Total	6.580E-003	19				

3.3 Physico-chemical – thermal characterization of VAP emulsion

The desirability ramp function graph (Figure 4) shows some of the quality control parameters of VAP emulsion as obtained from the physico-chemical studies. Particle size (droplet size) and polydispersity index (PDI) of the optimized and statistically validated formulation were found to be in the range of 940-942 nm and 0.41 respectively. The maximal encapsulation efficiency (EE) ranged from 98.2- 98.8%, however for the optimized batch it was found to be 98.8%.

The stability of the emulsion system is influenced by Particle size, PDI value and Zeta potential (value ranges from +100 mV to – 100mV for the particles). The value of PDI is correlated to particle size distribution and its value ranges from 0-1. The physical stability of the emulsion is more enhanced with narrow particle size distribution and the PDI value tending towards zero. In an emulsion system, particle size can be reduced by increasing the homogenization speed and time, altering the concentrations of the additives or excipients. The VAP emulsion system with particle size range of 940-942 nm (narrow size distribution) and PDI value of 0.41 gave significant physical stability. The optimized formulation showed no signs of destabilization after storage at normal room temperature for seven days. VAP emulsion showed a zeta potential (ζ) value in the range of 31.25 to -32.01 mv as measured in triplicate. The higher (ζ) value of the VAP emulsion (-31.25 to -32.01 mv) showed the high

dispersibility of VAP droplets between the aqueous and organic phases that aided in stabilizing the emulsion system; also supported by corroborative researches [20, 24].

The vitamin A content in VAP emulsion was determined to be 88.45±0.03% as measured in triplicate.

Table 3. Encapsulation efficiency (response 3) for encapsulation of vitamin A analyzed by ANOVA.

Source	Sum of squares	df	Mean square	F value	p-value Prob>F	
Model	12.80	13	0.98	2.572E+005	<0.0001	*Significant*
Linear mixture	8.58	3	2.86	7.467E+005	<0.0001	
AB	3.24	1	3.24	8.474E+005	<0.0001	
AC	0.52	1	0.52	1.367E+005	<0.0001	
AD	0.30	1	0.30	78824.07	<0.0001	
BC	1.10	1	1.10	2884E+005	<0.0001	
BD	1.08	1	1.08	2.829E+005	<0.0001	
CD	3.32BE-003	1	3.32BE-003	869.43	<0.0001	
ABC	1.15	1	1.15	2.991E+005	<0.0001	
ABD	1.55	1	1.55	4.061E+005	<0.0001	
ACD	3.533E-003	1	3.533E-003	922.86	<0.0001	
BCD	1.668E-004	1	1.668E-004	43.58	0.0006	
Residual	2.297E-005	6	3.82BE-006			
Lack of fit	2.297E-005	1	2.297E-005			
Pure error	0.000	5	0.000			
Cor Total	12.80	19				

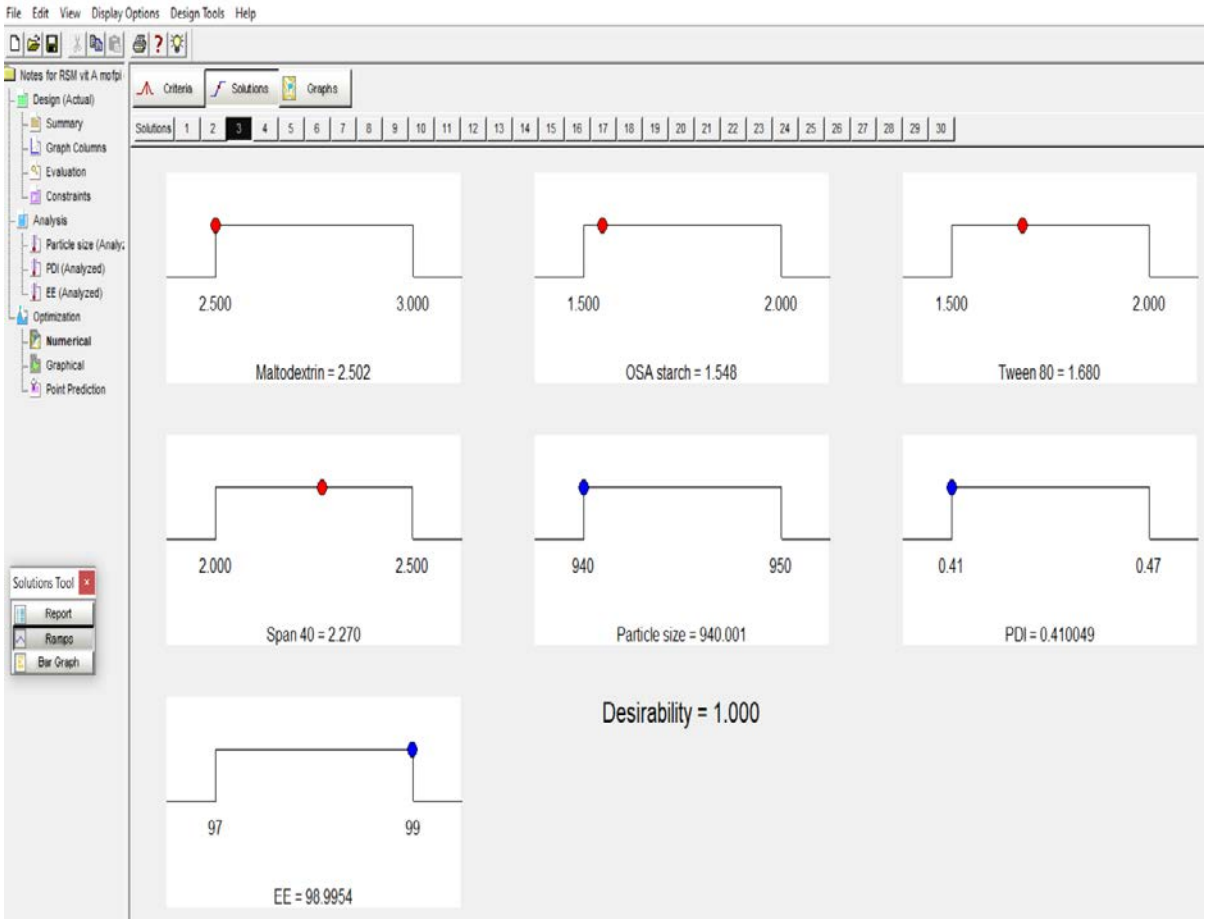

Fig. 4. Representative desirability ramp function graphs for final optimization of process parameters for vitamin A encapsulation

Regarding rheological studies, the optimized VAP emulsion showed Newtonian flow over the shear rate, thus its viscosity was measured at a fixed shear rate (10s-1). Viscosity of VAP emulsion was determined to be 1.561±0.03 as measured in triplicate. Interfacial tension is also a crucial parameter that plays an important role in attainment of smaller particle/droplet size during homogenization and is proportional to the deforming force or disruption of droplets from the homogenization zone. The interfacial tension of the optimized formulation was determined to be 22.6 mNm−1.

A comparative lower interfacial tension imparts transparency to the emulsion. However the optimized VAP emulsion was yellowish white obviously because of vitamin A. Interfacial tension alone can't ensure emulsion stability; droplet formation and coalescence combination are required for the same [20, 25]. Reduction in interfacial energy helps to lower the interfacial tension and increase the stability of the emulsion system. Amongst different recommended methods, surfactants find applications in lowering the interfacial tension. But VAP emulsion has been formulated for fortification purposes where over use of surfactants is likely to compromise with final safety aspects. Thus use of any excipients (e.g. emulsifiers, stabilizers) in the developed formulation was done considering the recommended dietary allowance (RDA) and regular daily intake (RDI) values.

The stability of the optimized samples of VAP emulsion was studied by keeping in different measuring cylinders closed with aluminum foil for 7 days in undisturbed condition. The VAP emulsion didn't show any changes in color, consistency or physical appearance. Thus the optimized process parameters were effective in preparing a stable VAP emulsion. The initial vitamin A content in VAP emulsion was determined to be 88.45±0.03%. After storing the formulation for seven days under the conditions of accelerated stability studies (55oC, 75% RH and 75oC, 80% RH respectively), the vitamin A content was found to be 88.07±0.05% and 86.01±0.04% respectively. Thus high temperature storage conditions (75oC, 80% RH) may likely reduce the vitamin A content.

In LT-DSC studies, vitamin A in VAP emulsion showed small endothermic peaks at -7^{o}C, 12^{o}C and 18^{o}C and a sharp peak at 30^{o}C which gradually disappeared with time. This is probably due to well dispersion of vitamin A in the emulsion system. Other components in VAP emulsion like vitamin E showed flat thermogram and other emulsifiers and thickeners e.g. maltodextrin, OSA modified starch etc showed small endothermic peaks at 48^{o}C and 58^{o}C that gradually disappeared at above 62^{o}C due to probable dispersion in the emulsion system giving further almost flat thermogram from 64^{o}C to 88^{o}C.

4. Conclusions

The optimized procedure for microencapsulation of Vitamin A emulsion by emulsification technique was found to be effective ensuring high encapsulation efficiency and stability. Successful fortification greatly depends on the stability and bioavailability of the fortificants. VAP emulsion was intended for vitamin A fortification. Dairy and non dairy beverages of plant origin and allied products are very suitable fortifying vehicles. The morphological appearance of the VAP emulsion was suitable for fortification in such beverages. Further researches are in progress for fortifying encapsulated VAP in the form of micronized emulsion in a suitable fortifying vehicle.

5. References

[1] Sauvant P, Cansell M, Sassi AH, Atgié C. Vitamin A enrichment: Caution with encapsulation strategies used for food applications. Food Research International. 2012;46: 469–79. 10.1016/j.foodres.2011.09.025.

[2] Mujica-Álvarez J, Gil-Castell O, Barra PA, Ribes-Greus A, Bustos R, Faccini M, Matiacevich S. Encapsulation of Vitamins A and E as Spray-Dried Additives for the Feed Industry. Molecules. 2020; 25: 1357. doi:10.3390/molecules25061357.

[3] Gangurde AB, Amin PD. Microencapsulation by Spray Drying of Vitamin A Palmitate from Oil to Powder and Its Application in Topical Delivery System. Journal of encapsulation and adsorption sciences. 2017; 7: 10-39. https://doi.org/10.4236/jeas.2017.71002.

[4] Sachdeva B, Kaushik R, Arora S, Khan A. Effect of processing conditions on stability of native vitamin A and fortified retinol acetate in milk. International Journal for Vitamin and Nutrition Research. 2019; 1–10. https://doi.org/10.1024/0300-9831/a000617

[5] Gonçalves A, Estevinho BN, Rocha F. Microencapsulation of vitamin A: a review. Trends in Food Science & Technology. 2016; 51: 76-87. http://dx.doi.org/10.1016/j.tifs.2016.03.001

[6] Klemm Rolf DW, West KP, Palmer Jr., AC, Johnson Q, Randall P, Ranum P, Northrop-Clewes C. Vitamin A fortification of wheat flour: Considerations and current recommendations. Food and Nutrition Bulletin. 2010; 31 (1): S47-S61.

[7] Hombali AS, Solon JA, Venkatesh BT, Nair NS, Peña-Rosas JP, Cochrane Public Health Group. Fortification of staple foods with vitamin A for vitamin A deficiency. Cochrane Database of Systematic Reviews. 2019; (5): CD010068. doi: 10.1002/14651858.CD010068.pub2

[8] Tanglao EJ, Kumar ABN, Noriega RR, Punzalan ME, Marcelo P. Development and physico-chemical

characterization of virgin coconut oil-in-water emulsion using polymerized whey protein as emulsifier for Vitamin A delivery. MATEC Web of Conferences. 2019; 268: 01002. https://doi.org/10.1051/matecconf/201926801002

[9] Carlotti ME, Sapino S, Trotta M, Battaglia L, Vione D, Pelizzetti E. Photostability and Stability over Time of Retinyl Palmitate in an O/W Emulsion and in SLN introduced in the Emulsion, Journal of Dispersion Science and Technology. 2005; 26:2:125-138, DOI:10.1081/DIS-200045403

[10] Jun SH, H Kim, Lee H, JE Song, SG Park, Kang NG. Synthesis of Retinol-Loaded Lipid Nanocarrier via Vacuum Emulsification to Improve Topical Skin Delivery. Polymers 2021; 13: 826. https://doi.org/10.3390/polym13050826

[11] Carafa M, Marianecci C, Salvatorelli M, Marzio JDi, Cerreto F, Lucania G, Santucci E. Formulations of retinyl palmitate included in solid lipid nanoparticles: characterization and influence on light-induced vitamin degradation. Journal of Drug Delivery Science and Technology. 2008; 18 (2): 119-124.

[12] Mart´ınez-Sancho C, Herrero-Vanrell R, Negro S. Vitamin A palmitate and aciclovir biodegradable microspheres for intraocular sustained release. International Journal of Pharmaceutics. 2006; 326 :100–106. doi:10.1016/j.ijpharm.2006.07.010

[13] AlZahabi SMA. Vitamin a palmitate-loaded NLCs vs. SLN for cosmetic application with a study of their controlled release, skin permeation and characterization. American University in Cairo, Master's Thesis. AUC Knowledge Fountain. 2017. https://fount.aucegypt.edu/etds/549

[14] Errico C, Goñi-de-Cerio F, Alderighi M, Ferri M, Suarez-Merino B, Soroka Y, Frušic´-Zlotkin M, Chiellini F. Retinyl palmitate–loaded poly(lactide-co-glycolide) nanoparticles for the topical treatment of skin diseases. Journal of Bioactive and Compatible Polymers. 2012; 27(6): 604 –620. DOI: 10.1177/0883911512461107.

[15] Boskabadi M, Saeedi M, Akbari J, Morteza-Semnani k, Hashemi SMH, Babaei A. Topical Gel of Vitamin A Solid Lipid Nanoparticles: A Hopeful Promise as a Dermal Delivery System. Advanced Pharmaceutical Bulletin. 2021; 11(4): 663-674. doi:10.34172/apb.2021.075.

[16] De B, Bhandari K, Katakam P, Saha G, Goswami TK. Turmeric black tea as a multimodal theronostic dietary adjuvant aiding neuroprotection and ameliorating hypertension. MOJ Current Research & Reviews. 2019; 2(5): 1-10.

[17] De B, Bhandari K, Singla RK, Katakam P, Samanta T, Kushwaha DK, Gundamaraju R, Mitra A. Chemometrics optimized extraction procedures, phytosynergistic blending and in vitro screening of natural enzyme inhibitors amongst leaves of Tulsi, Banyan and Jamun. Pharmacognosy Magazine. 2015; 11(44): 522-532.

[18] Bhandari K, Singla RK, De B, Ghosh BC, Katakam P, Khushwaha DK, Gundamaraju R, Sen G, Saha G, Mitra A, Mitra A. Chemometrics Based Extraction of polyphenlolics from fresh tea leaves and processed tea showing in-silico docking and anti-oxidative theronostic dietary adjuvant in Alzheimer. Indo Global Journal of Pharmaceutical Sciences. 2015; 5(3): 171-191.

[19] De B, Bhandari K, Katakam P, Goswami KT. Development of a standardized combined plant extract containing nutraceutical formulation ameliorating metabolic syndrome components. SN Applied Sciences. 2019; 1:1484.

[20] De B, Goswami TK. Chemometrics optimized microencapsulation of ergocalciferol using Emulsification technique and its physico-chemical characterization. Applied Food Research. 2022; 2: 1-8. https://doi.org/10.1016/ j.afres.2022.100157

[21] Mujica-Álvarez J, Gil-Castell O, Barra PA, Ribes-Greus A, Bustos R, Faccini M, Matiacevich S. Encapsulation of Vitamins A and E as Spray-Dried Additives for the Feed Industry. Molecules. 2020; 25: 1357. doi:10.3390/molecules25061357

[22] Gangurde AB, Ali MT, Pawar JN, Amin PD. Encapsulation of vitamin E acetate to convert oil to powder microcapsule using different starch derivatives. Journal of Pharmaceutical Investigation. 2017; 47: 559–574.

[23] Kamangar T, Fawz AB. Spectrophotometric Determination of Vitamin A in Oils and Fats. Journal of Association of Official Analytical Chemists. 1978; 61 (3):753- 755.

[24] de Souza SL, de Faria JT, Amorim ML, de Araújo JM, Minim LA, dos Reis Coimbra JS, de Carvalho Teixeira AVN, de Oliveir EB. Rheological and Physicochemical Studies on Emulsions Formulated with Chitosan Previously Dispersed in Aqueous Solutions of Lactic Acid. Food Biophysics. 2017; 12(1): 109-118. DOI: 10.1007/s11483-017-9469-4

[25] Schroën K, de Ruiter J, Berton-Carabin C. The Importance of Interfacial Tension in Emulsification: Connecting Scaling Relations Used in Large Scale Preparation with Microfluidic Measurement Methods. Chemical Engineering Journal. 2020; 4(4): 63. DOI: 10.3390/chemengineering4040063