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DESIGN AND DEVELOPMENT OF VITAMIN 'A' STABILIZATION BY DIFFERENT TECHNIQUE

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ABSTRACT

Vitamin A Palmitate (VAP) contains retinol and palmitic acid, which are essential to the body. But it is a light-sensitive molecule that undergoes degradation when exposed to UV light. The purpose of this study was to prepare a stabilised VAP powder using Spray drying and the encapsulation technique. For spray drying, an emulsion of VAP was prepared using maize starch and maltodextrin with tween 80 as an emulsifier and the resulting emulsion was spray dried. For encapsulation, VAP was mixed with MCC and sorbic acid as a preservative and the mixture was lyophilized. The stabilised powder contains 35% VAP and was produced using different concentrations of wall materials. The prepared powder was evaluated for their physical properties, drug content, *in-vitro* drug release and SEM study. The result showed that the obtained powder is nearly spherical in shape, with a particle size range of 1–14 µm. The drug content of different batches was found to be within an acceptable range. The drug release study showed 87.41% to 95.8% of drug release from stabilised powder at the end of 60 minutes. The formulations were kept for a 3-month stability study as per ICH guidelines and found to be stable.

KEYWORDS: Vitamin A Palmitate, Spray drying, Encapsulation, Lyophilization, Stability studies.

INTRODUCTION

Vitamins are vital micronutrients that are involved in many biological functions in the body. An adequate intake of Vitamins is known to maintain normal health and immunity, help regulate metabolism in the body and in some cases to prevent chronic diseases.

Vitamins are categorised into two types based on their solubility in water or fat.

- Fat soluble Vitamins A, D, E and K.
- Water-soluble Vitamins B and C.

Vitamin A is an essential nutrient needed in small amounts for the normal functioning of the visual system and the maintenance of cell function for growth, epithelial integrity, red blood cell production, immunity and reproduction.[1]

Recommended Dietary Allowances (RDAs) for Vitamin A.

Age	Male	Female	Pregnancy	Lactation
Birth to 6 months	400 mcg RAE	400 mcg RAE		
7-12 months	500 mcg RAE	500 mcg RAE		
1-3 years	300 mcg RAE	300 mcg RAE		
4-8 years	400 mcg RAE	400 mcg RAE		
9-13 years	600 mcg RAE	600 mcg RAE		
14-18 years	900 mcg RAE	700 mcg RAE	750 mcg RAE	1200 mcg RAE
19-50 years	900 mcg RAE	700 mcg RAE	770 mcg RAE	1300 mcg RAE
54+ years	900 mcg RAE	700 mcg RAE		

RAE: Retinol Activity Equivalents[2]

Vitamin A Deficiency[3]

It causes

a) Night blindness.
b) Xeropthalmia or dry eyes.
c) Reproductive functions may also be affected by Vitamin A deficiency.
d) Compromised Immune system.
e) Poor dental Health.

Causes for Vitamin A unstability

Vitamin A is sensitive to light, particularly ultraviolet (UV) light. When Vitamin A is exposed to light, the

energy from the light can break down the double bonds in the molecule, resulting in the formation of free radicals. These reactions can cause the degradation of Vitamin A and reduce its effectiveness.

Exposure to light can cause the degradation of Vitamin A, leading to a loss of its biological activity. This sensitivity is due to the chemical structure of Vitamin A, which contains a conjugated double bond system that can undergo photochemical reactions.[4]

Vitamin A is sensitive to heat and can undergo degradation when exposed to high temperatures for extended periods. Heat can cause the breakdown of the molecular structure of vitamin A, leading to a loss of its nutritional value. The exact temperature and duration required to degrade Vitamin A may vary, but it generally begins to degrade significantly at temperatures above 60°C. So, we aim to prepare Vitamin A in a stabilised form that has a reasonably high shelf life, using different techniques such as Spray drying and Encapsulation.

MATERIALS AND METHODS

For spray drying technique

Vitamin A Palmitate, Vitamin E as anti-oxidant, Colloidal silicon dioxide as glidant, Maize starch coating agent, Maltodextrin as bulking agent, Tween 80 as emulsifier.

For encapsulation technique

Vitamin A Palmitate, Vitamin E as anti-oxidant, Micro crystalline cellulose as bulking agent, Sorbic acid and sodium benzoate as preservatives, Aerosil as glidant, Alginate as emulsifyingagent, Tween 80 as emulsifier.

Preformulation studies

- Organoleptic properties
- Solubility analysis:10mg of VAP dissolve in various solutions like IPA, ethanol, methanol, chloroform, ethyl ether.

Determination of λ max

1mg of VAP were dissolved in 10ml of IPA and the maximum absorption was analysed between 200-400 nm using UV-Visible spectrophotometer.

Preparation of Standard calibration curve of VAP

100mg of VAP were dissolved in 100ml IPA solution to get the concentration of 1mg/ml. Fromthe above solution pipette out 10ml and make up to 100ml using IPA solution to get 100µg/ml concentration. And pipette out 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 and 0.7 ml and make up to 10ml using IPA to get 1, 2, 3, 4, 5, 6 and 7 µg/ml and analysed against a blank (IPA) by using the UV - Visible spectrophotometer.

Compatibility study using FT-IR

In the preparation of stabilised Vitamin A, polymer may interact as they are in close contact with each other, which could lead to the instability of drug. FTIR spectroscopy helps to identity of the drug and polymer interaction. The pure drug, pure polymer, physical mixture of drug, polymer and other excipients were prepared and scanned from 4000-400cm^{-1} in FTIR spectrophotometer the IR spectrum of pure VAP and formulated VAP powder were recorded by FTIR spectrophotometer.[5]

Method for preparation of stabilized VAP powder Preparation of stabilised Vitamin A by spray dryer technique.

Sl no	Ingredients	F 1	F 2	F 3
1	Vitamin A palmitate	35	35	35
2	Vitamin E	2	2	2
3	Colloidal silicon dioxide	20	20	20
4	Maize starch	20	15	10
5	Maltodextrin	18	23	28
6	Tween 80	5	5	5

Quantities % w/w

Vitamin A Palmitate was heated to 50^{O}C, tween 80 and vitamin E was mixed and kept aside. Water was boiled and maintained at 60-65^{O}C. Colloidal silicon dioxide, maize starch and maltodextrin was mixed and kept aside. VAP and tween 80 mixture was added to water with constant stirring to o/w emulsion. To the obtained emulsion, colloidal silicon dioxide, maize starch and maltodextrin were added with constant stirring and required amount of water was added later. Obtained solution is spray dried with inlet temperature of 110-130^{O}C and outlet temperature of 55-60^{O}C at 12,000 rpm. The product obtained is stored in sealed container in a black cover.

Preparation of stabilised VAP powder by encapsulation

Dissolve sorbic acid in water, boil and add sodiumbenzoate, alginate and mix well, this solution was added to the MCC. VAP, Vitamin E and Tween 80 was mixed separately and kept aside. The obtained emulsion of VAP, VE and Tween 80 were added to the MCC mixture with constant stirring, followed by addition of Aerosil. Lyophilize the obtained product. Check the moisture content of the product after 5-6 hrs, continue lyophilization till the moisture content is not more than 3%.

Sl no.	Ingredients	F1	F2	F3
1	Vitamin A palmitate	35	35	35
2	Vitamin E	2	2	2
3	Micro crystalline cellulose (MCC)	50	55	60
4	Sorbic acid	0.5	0.5	0.5
5	Sodium benzoate	0.5	0.5	0.5
6	Aerosil	3	3	3
7	Alginate	3	3	3
8	Tween 80	1	1	1

EVALUATION OF THE PREPARED STABILISED VAP POWDER

$$\text{Percentage yield} = \frac{\text{weight of prepared VAP powder}}{\text{total weight of drug and polymer}} \times 100$$

DRUG CONTENT

HPLC analysis[6]:- Chromatographic conditions

The separation was carried out on RP- HPLC system (Shimadzu, UV-1900i Japan) with HPLC pump, photo diode array (PDA) detector, LabSolutions software and Luna, 5u C18, column (250mmx4.6mm)

Preparation of mobile phase for VAP

The mobile phase was prepared by the mixture of Methanol, Acetonitrile and Water in the ratioof 750 : 225 : 25 v/v (HPLC grade) and was filtered through 0.45 µm membrane filter (Milli- pore, USA) and degassed.

Preparation of standard VAP solution

Accurately weighed and transferred about 100 mg of VAP into a 50 ml clean, dry amber coloured volumetric flask and made up to the volume with hexane to get concentration 2 mg/ml.From the above solution 1ml was further diluted to 50ml with methanol.

Preparation of sample VAP solution

Equivalent to 100mg of weighed VAP powder were suspended in the 50 ml volumetric flask and made up to the volume with hexane. From the above solution 1ml was further diluted to 50 ml with methanol.

Procedure

Mobile phase was pumped into the column at a flow rate of 2.0 ml/min. The volume of the injection loop was set to 20 µl prior to the auto-injection of standard and sample solution and the column was equilibrated for at least 30 min with the mobile phase flowing through the system. The detection was monitored at 325 nm for VAP and the run time was 15 min. Recorded the area of the chromatogram.

The drug content was calculated by using the formula,

$$\text{Assay} = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Standard weight}}{\text{Standard dilution}} \times \frac{\text{Sample dilution}}{\text{Sample weight}} \times \text{Standard purity}$$

Micromeritic study

The flow property of the powder was studied by determining the parameters like angle of +repose, bulk density, tapped density, Carr's index and Hausner's ratio.

- **Angle of repose:** tan θ= h/r or θ=tan-1 (h/r)where, h = height of pile

r = radius of the base of the pile

θ= angle of repose

- $\text{Bulk density} = \frac{\text{weight of the VAP powder } (W)}{\text{Initial volume occupied by the powder } (V0)}$
- $\text{Tapped density} = \frac{\text{weight of the powder } (W)}{\text{final volume occupied by the powder } (vf)}$
- $\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$
- $\text{Carr's index } C_i = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100$

Scanning electron microscopy (SEM)[7]

To evaluate physical surface and morphology of stabilized powder like size and shape was analysed using scanning electron microscope.

IN-VITRO DRUG RELEASE STUDY[8]

The *in-vitro* dissolution studies were carried out using USP type - II Dissolution apparatus. VAP stabilised powder was filled in tea bag and tea bag were placed in dissolution apparatus containing 900ml 7.4 pH phosphate buffer which was maintained at 37±0.5^{O}C and at a stirring speed of 50 rpm. 5ml samples were withdrawn at predetermined time intervals and same volume of fresh medium was replaced into the basket. Sample was withdrawn at time intervalsof 5, 10, 20, 30, 40, 50 and 60 min. The concentration of drug released was estimated by usingUV spectrophotometer at λ max 325nm.

STABILITY STUDIES

In order to determine the change in the parameters like physical appearance, drug content, *in- vitro* drug release profile on storage, stability studies of optimized batch were carried out at short term and accelerated storage condition at temperature 25±2^{0} C with 60±5% RH and 40±2^{O}C with 75±5% RH in a stability chamber for 90 days. Sample were withdrawn after 30, 60, 90 days evaluated for changes in physical appearances and drug content.

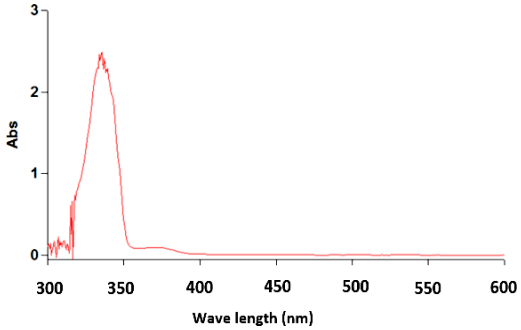
RESULTS AND DISCUSSION

Pre-formulation studies of VAP.

Organoleptic characteristics & Solubility of VAP.

Properties	Reported		Observed
Appearance	Yellow viscous oil		Yellow viscous oil
Odour	Odour		Mild odour
Solubility	Ethanol	Soluble	Soluble
	Methanol	Soluble	Soluble
	IPA	Soluble	Soluble
	Chloroform	Soluble	Soluble
	Ethyl ether	Soluble	Soluble
	Water	Insoluble	Insoluble
	Glycerol	Insoluble	Insoluble

Determination of λ max



λ max of VAP in IPA

Solution of VAP (100 µg/ml) was prepared using IPA and this solution was scanned for absorbance 200-800 nm using UV spectrophotometer. As shown in fig. peak was obtained at 325 nm. The absorption maximum (λmax) was found 325 nm. This value was selected for restof the UV spectrophotometric analysis.

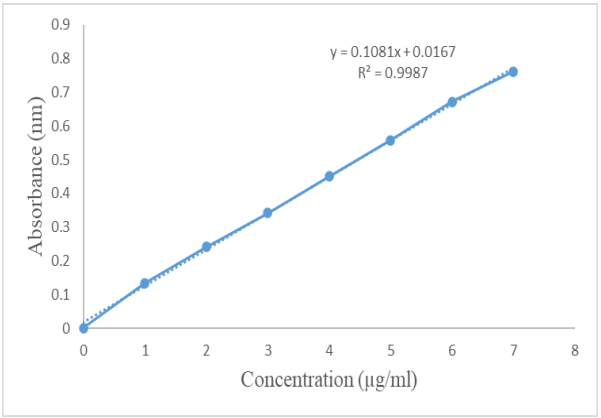
Standard calibration plot

Sl. no	Concentration (µg/ml)	Absorbance ± SD*
1	0	0
2	1	0.134±0.001
3	2	0.241±0.004
4	3	0.342±0.002
5	4	0.451±0.001
6	5	0.558±0.003
7	6	0.672±0.002
8	7	0.761±0.002

*All the Values represents are mean of 3 readings (n=3)

±SD- Standard deviation

Standard calibration data of VAP

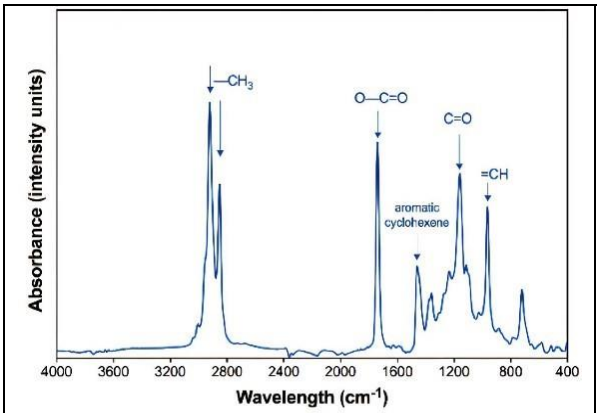


Standard calibration plot of VAP.

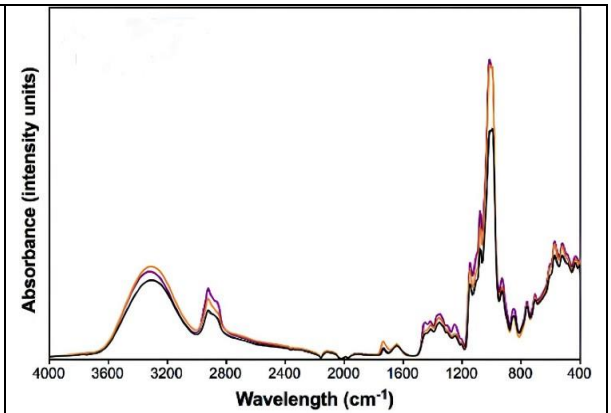
The drug solution of 1µg/ml - 7µg/ml was prepared using IPA and absorbance measured using UV spectrophotometer at the absorption maximum (λ max) 325 nm. The obtained absorbance data is plotted against the concentration of drug solution. Absorbance value remained linear and obeyed Beer's Lamberts law in the range of 0-7µg/ml with the slope value as y = 0.1081x + 0.0167 and R2 value of 0.9987.

Compatibility studies using FT-IR.

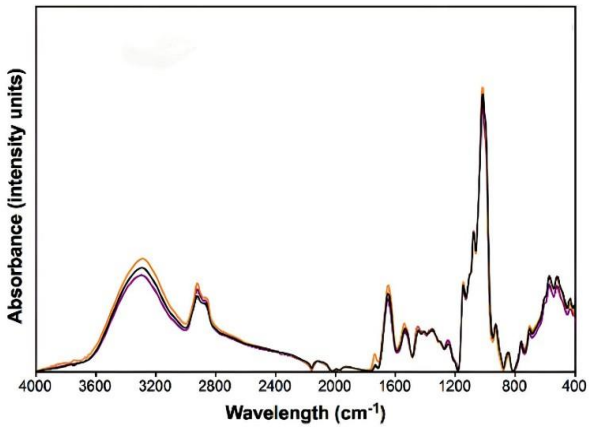
Functionalgroups	Wavelength from 400 to 4000 cm^{-1}			
	Vitamin palmitate	Vitamin E	Spray dried product	Encapsulated product
CH_3 stretching	2922	2864	-	-
C=O stretching	1739	-	1725	1640
-CO stretching	1350	1355	1372	1367
CH	2900	-	2905	2910
OH	-	3473	3475	3451
CH2	-	1422	1428	1437



FTIR spectra of pure Vitamin A palmitate.



FTIR spectra of spray dried VAP powder.



FTIR spectra of encapsulated VAP powder.

The results of the IR spectrum of excipients and the drug VAP showed the presence of characteristic peaks very similar to those of the reference peaks reported previously. While theIR spectra of the drug and the drug-loaded particles showed no absence of new peaks or disappearance of the existing peak, which shows that there was no covalent interaction between the VAP and excipients and furthermore, the polymer did not alter the performance characteristics of drugs.

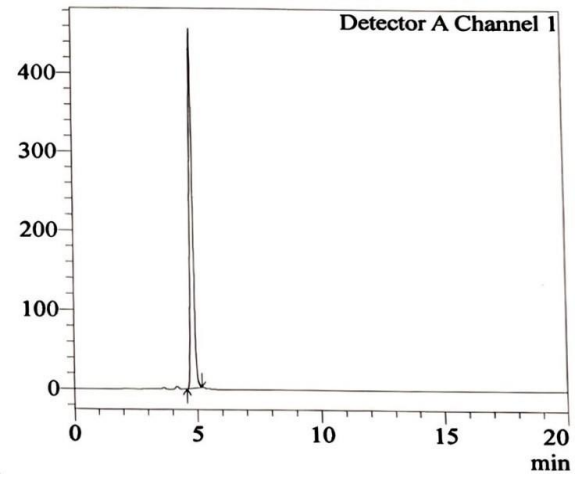
Evaluation of VAP stabilised powder

Percentage yield of VAP stabilised powder

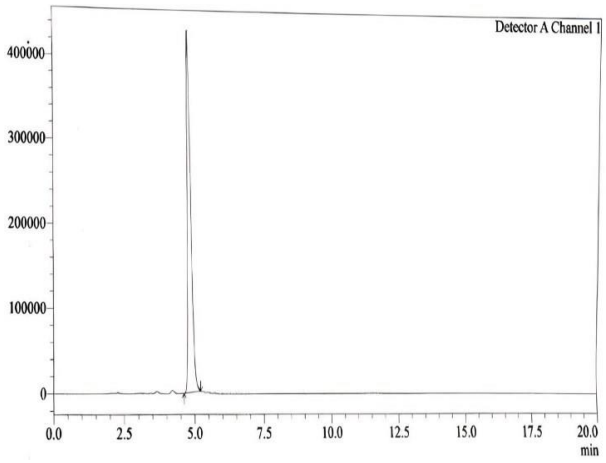
Sl no.	Formulation	% yield w/w
In spray dried technique		
1	F1	80.72
2	F2	82.21
3	F3	78.68
In encapsulation technique		
1	F1	91.26
2	F2	95.05
3	F3	92.10

DRUG CONTENT DETERMINATION.

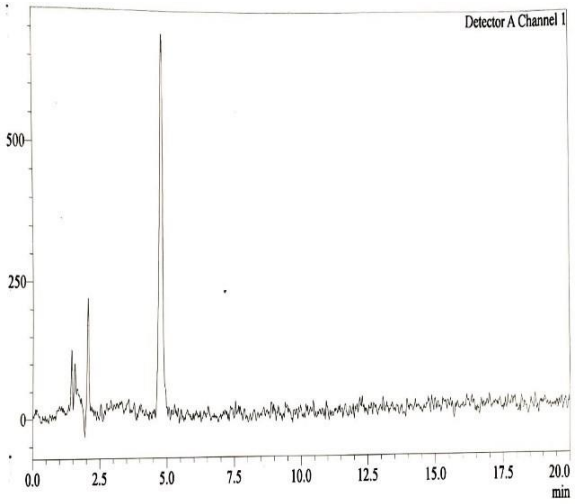
The drug content is determined using HPLC.



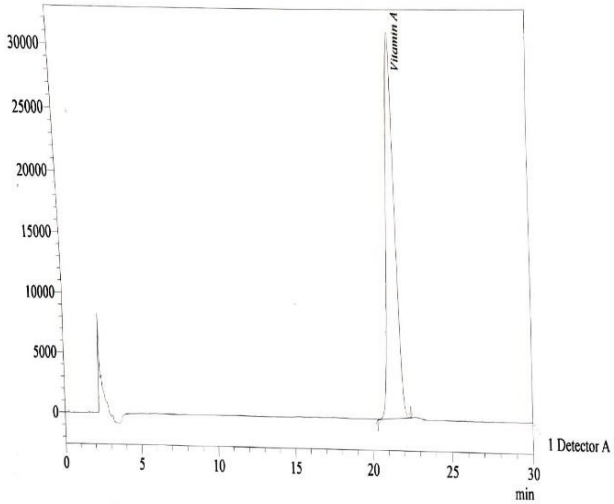
HPLC graph of Spray dried Formulation 1



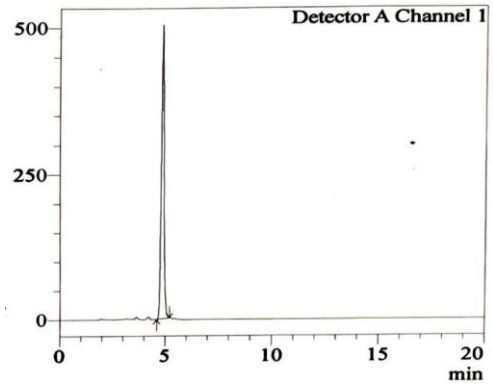
HPLC graph of Spray dried Formulation 2



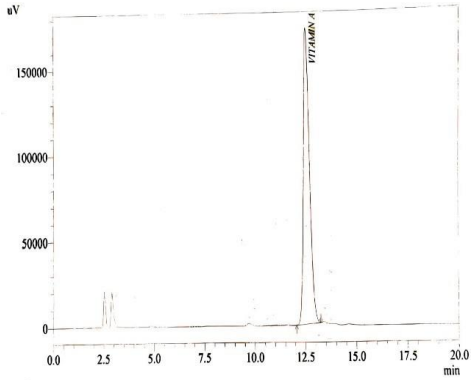
HPLC graph of Spray dried Formulation 3



HPLC graph of Encapsulated Formulation 1.



HPLC graph of Encapsulated Formulation 2



HPLC graph of Encapsulated Formulation 3

Formulations	Area of principle peak	Weight of sample (mg)	% Assay
Spray dried technique			
F1	3480412	100.02	96.64
F2	3486872	99.59	97.41
F3	3479363	100.5	96.23

In encapsulation technique			
F1	3497253	99.57	97.31
F2	3584358	99.89	99.73
F3	3501213	99.38	98.01

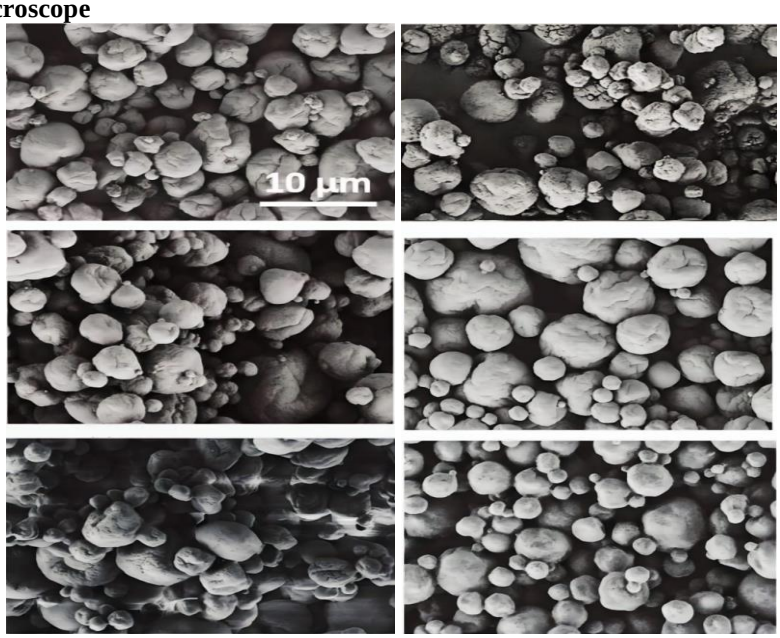
HPLC data of VAP stabilised powder.

The drug content of VAP was determined using HPLC in both techniques. The drug content was found to be in the range of 96.23 to 97.41% in the spray drying technique and 97.31 to 99.73% in encapsulation technique.

Micromeritic study

Formulation code	Angle of repose (θ^O)	Bulk Density (gm/ml)	TappedDensity (gm/ml)	Carr's index(%)	Hausner Ratio
In spray drying technique					
F1	38.65±0.24	0.253±0.02	0.322±0.01	21.42±0.4	1.27±0.02
F2	37.95±0.31	0.243±0.01	0.307±0.01	20.84±0.2	1.26±0.04
F3	39.35±0.27	0.240±0.01	0.307±0.03	21.82±0.5	1.27±0.02
In encapsulation technique					
F1	32.61±0.18	0.465±0.02	0.540±0.02	13.83±0.6	1.16±0.03
F2	30.54±0.35	0.444±0.03	0.513±0.02	13.33±0.3	1.15±0.02
F3	31.38±0.23	0.416±0.02	0.487±0.01	14.57±0.4	1.17±0.02

Scanning electron microscope

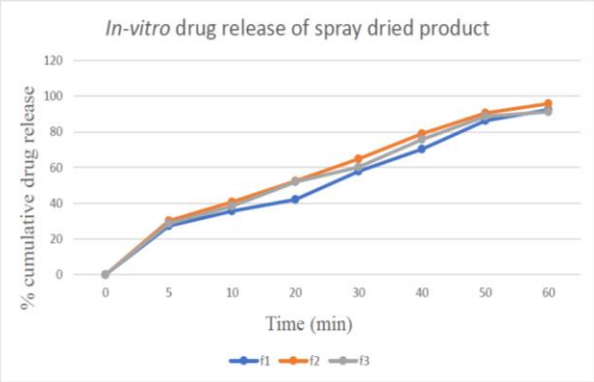


SEM images of stabilised VAP powder by spray drying technique and encapsulated.

The shape and surface morphology of the prepared VAP powder were observed by scanning electron microscopy. Scanning electron microscopy reveals that the stabilised VAP has a semi-spherical shape. Particle size distribution with a more frequent diameter in the range from 1 to 14 µm.

***In vitro* drug release study**

Time (min)	Cumulative % drug release		
	F1	F2	F3
0	0	0	0
5	27.3±0.12	30.2±0.21	28.7±0.20
10	35.6±0.15	40.7±0.15	38.4±0.11
20	42.1±0.13	52.4±0.14	51.9±0.16
30	57.9±0.17	64.8±0.12	60.3±0.13
40	70.2±0.20	78.9±0.14	75.7±0.13
50	86.3±0.18	90.5±0.15	88.6±0.11
60	92.4±0.17	95.8±0.16	91.2±0.20

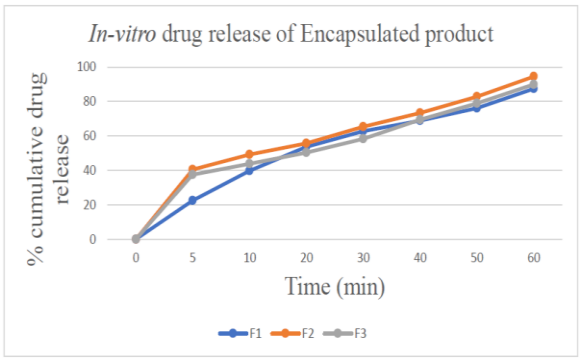


Percentage cumulative drug release data of stabilised VAP powder by spray drying technique from formulations F1, F2 and F3.

***In-vitro* drug release profile of VAP spray dried product**

The formulations F1, F2 and F3 containing spray dried VAP powder showed percentage drug realease of 92.4%, 95.8% and 91.2% respectively. In this the formulation F2 showed a better drug realease of 95.8% at the end of 60minutes.

Time (min)	Cumulative % drug release		
	F1	F2	F3
0	0	0	0
5	22.46±0.16	40.37±0.18	37.52±0.12
10	39.63±0.18	49.24±0.12	43.79±0.16
20	53.59±0.21	55.61±0.16	50.36±0.18
30	62.74±0.16	65.34±0.19	58.31±0.14
40	68.84±0.13	73.36±0.15	69.36±0.16
50	76.12±0.21	82.87±0.17	78.83±0.21
60	87.41±0.15	94.48±0.12	89.93±0.14



Percentage cumulative drug release data of stabilised VAP powder by encapsulation technique from formulations F1, F2 and F3.

***In-vitro* drug release profile of VAP encapsulated product**

The formulations F1, F2 and F3 containing encapsulated VAP powder showed percentage drug realease of 87.41%, 94.48% and 89.93% respectively. In this the formulation F2 showeda better drug realease of 95.8% at the end of 60minutes.

Stability studies

Stability studies of stabilised VAP powder of spray dried product.

Time (days)	Temperature & Humidity	Drug content (%)		
		F1	F2	F3
0	-	96.64	97.41	96.23
30	**At 25±2^{0}C, 60±5% RH**	95.85	97.02	95.26
	At 40±2^{0}C, 75±5% RH	95.51	96.93	95.11
60	**At 25±2^{0}C, 60±5% RH**	94.06	96.91	94.82
	At 40±2^{0}C, 75±5% RH	93.87	96.85	94.53
90	**At 25±2^{0}C, 60±5% RH**	93.51	96.17	93.58
	At 40±2^{0}C, 75±5% RH	93.04	95.97	93.12

Stability studies of stabilised VAP powder of encapsulated product.

Time (days)	Temperature & Humidity	Drug content (%)		
		F1	F2	F3
0	-	97.31	99.73	98.01
30	**At 25±2^{0}C, 60±5% RH**	96.95	98.62	97.35
	At 40±2^{0}C, 75±5% RH	96.82	98.47	97.05
60	**At 25±2^{0}C, 60±5% RH**	95.72	97.59	96.58
	At 40±2^{0}C, 75±5% RH	95.47	97.22	96.11
90	**At 25±2^{0}C, 60±5% RH**	94.92	97.01	94.98
	At 40±2^{0}C, 75±5% RH	94.81	96.85	94.77

CONCLUSION

Spray drying and encapsulation techniques were used to stabilise Vitamin A. Three formulations were prepared by both techniques and evaluated. Micromeritic studies revealed that the prepared Vitamin A powder exhibited good flow. Scanning electron microscopy reveals that the stabilised VAP has a semi-spherical shape. The *in-vitro* drug release studies show that the obtained cumulative drug release (CDR) was found to be significant. The short- term stability studies of both technique products indicate that there are no significant changes in physical appearance and drug content after 90 days of storage. Altogether, the proposed techniques are feasible for the stabilisation of vitamin A and protection against degradation.

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