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Study of the Effect of Preparation and Formulation Factors on the Stability of Vitamin C Microencapsulated by Solvent-Evaporation Method

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Abstract:- This research studied the effect of formulation and processing variables on the stability of vitamin C or Ascorbic Acid (AA) encapsulated in Eudragit RS100 microparticles prepared using a solvent evaporation technique. Microparticles ranged in size from 60 to 102 µm and encapsulation efficiencies between 25% and 44% were obtained. Microencapsulation significantly enhanced the stability of AA compared to the free AA in solution. AA shelf life was extended up to 23 days. Increasing AA concentration and using sucrose or Tween 80 in the outer phase further enhanced AA stability. The results suggest that microencapsulation method under the investigated conditions is a promising approach for protecting AA from degradation in aqueous solutions.

Keywords:- *Vit C, Shelf Life, Formulation, Microparticles, Solvent Evaporation.*

I. INTRODUCTION

Vitamin C (VIT C), also known as ascorbic acid (L-enantiomer, FIGURE 1), is a water-soluble vitamin with a chemical structure related to C6 sugars. It has six carbons and features an enediol group on carbons 2 and 3, making it the aldono-1,4-lactone of a hexonic acid [1]. Vitamin C plays a crucial role in collagen formation, a protein that provides structure to bones, cartilage, muscles, and blood vessels. Additionally, it acts as an antioxidant by donating electrons and preventing other molecules from being oxidized. As a potent water-soluble antioxidant, its effects have been demonstrated in numerous in vitro experiments [2]. It offers various benefits for the skin, including collagen synthesis, depigmentation, and antioxidant activity. As an antioxidant, it shields the skin by neutralizing reactive oxygen species (ROS) generated by sunlight exposure [3].

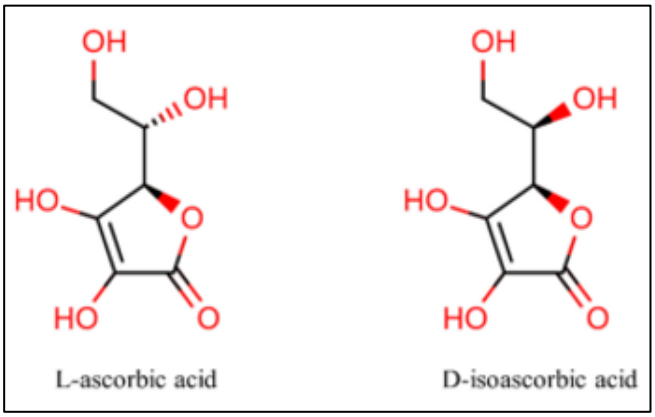

Fig 1 Structures of L-Ascorbic Acid and its Stereoisomer

Developing ascorbic acid products faces a significant hurdle: its high instability and reactivity. Light, heat, transition metal ions, and alkaline conditions can all trigger the reversible oxidation of ascorbic acid to dehydroascorbic acid (DHA). DHA then undergoes further irreversible hydrolysis to form 2,3-diketogulonic acid [3].

Fortunately, several strategies can address this issue. These include formulation-friendly derivatives like ascorbyl 6-palmitate, tetra-isopalmitoyl ascorbate, magnesium ascorbyl phosphate, sodium ascorbyl phosphate, ascorbyl 2-glucoside, ascorbyl 2-phosphate-6-palmitate, and 3-O-ethyl ascorbate, maintaining a low pH environment, using oxygen-impermeable packaging, incorporating electrolytes and other antioxidants and encapsulation.

Microspheres are solid, spherical particles with a size range of 1-1000 micrometers (typically 1-500 micrometers) used for controlled drug release and localized effects. They offer protection to the drug from chemical and enzymatic degradation, potentially reducing toxicity and improving therapeutic efficacy [4].

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There are various microencapsulation techniques for vitamin C as Spray Drying, Extrusion and solvent evaporation method. [1]. This research employed solvent evaporation method due to its versatility, ability to produce high-quality microparticles, and status as a relatively inexpensive, rapid, and easily scalable technology when compared to other microencapsulation techniques (justifying its industrial preference) [5]

The aim of this study was to study the effect of different variables (formulation and preparation) on the stability of Vit C encapsulated in Eudragit RS100 microparticles.

II. MATERIAL AND METHOD

A. Material

Vitamin C was purchased from Loba chemie, India. 2,6-dichorophenol: Indophenol Sodium was obtained from Tmmedia, India. Eudragit RS 100, alcohol poly vinyl PVA and saccharose were obtained from Sigma-Aldrich. Oxalic acid and tween 80 were of analytical grade.

B. Methods

➢ *Microparticles Preparation*

VIT C microparticles were prepared by double emulsion solvent-evaporation according to [6] with some modifications. Briefly, an aqueous solution of VIT C was vortexed (Classic Vortex mixer VELP Scientifica Srl_ Italy) in organic solution containing DCM and Eudragit RS 100 to form W1/O primary emulsion. This W1/O was then re-emulsified under magnetic agitation (A & E Lab (UK) Co., Ltd) in little volume of outer aqueous containing Tween or PVA to form W1/O/W2 double emulsion. The formed emulsion was transferred to a large volume of water containing Tween or PVA (W2) to allow evaporation of DCM. The formed microparticles were filtered, washed and air-dried. Different microparticles formulas were grouped in TABLE 1.

Table 1 Composition of Microparticles Preparation Under Different Conditions

	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8
Eudragit RS (g)	0.5	0.5	0.5	0.5	1	0.5	0.5	0.5
Tween 80 (%)	1%	2%	-	-	-	-	-	-
PVA (%)	-	-	1%	0.5%	1%	1%	1%	1%
Stirring duration 1 (min)	0.5	0.5	0.5	0.5	0.5	1	0.5	0.5
Sucrose (g)	-	-	-	-	-	-	-	1

➢ *Vit C Determination*

The bioactive Vit C was determined using 2,6-dichlorophenol: indophenol (DCPIP) method according to AOAC official method (1984) [7] with minor modification in which Metaphosphoric acid was substituted by oxalic acid (0.2%). Titration methods are cheap and don't request sophisticated equipment [8]. Diluted Vit C solutions from outer phases and encapsulated vit C were titrated by standardized solution DCPIP.

➢ *Microparticles Characterization*

- *Particle size*

Particle size was determined by optical calibrated microscope (A & E Lab (UK) Co., Ltd) according to [9]. The mean diameter was calculated according to the equation:

$$d=\frac{\in nidi}{\in ni}$$

- *Encapsulation Efficiency EE*

For EE determination, an amount of dry microparticles was dissolved in 3ml acetate ethyl and vitamin C was extracted in 5ml water under magnetic agitation (A & E Lab (UK) Co., Ltd) during 15 minutes. The encapsulated VIT C was calculated according the following:

$$EE\%=\frac{Practical\ amount}{Theorotiacl\ amount}*100$$

- *Vit C Stability Analysis Using Accelerated Stability Test*

Dry microparticles and eight closed tubes containing outer phase of each experiment were placed in two ovens (A&E Lab, UK) at two temperatures: 37° and 45° C for accelerated stability test [10, 11] . The microparticles and the tubes were stored in the dark and samples were taken periodically at 0, 7, 14, 21 and 28 days. For microparticles, VIT C was extracted in water and bioactive VIT C was determined as EE protocol determination.

An aqueous solution of Vit C without additives was used as a control.

- *Kinetic Modeling*

A zero-order model: $C=C_0-Kt$ (1) and first order model: $\ln C=\ln C_0-Kt$ (2) were fitted to determinate the kinetic of Vit C degradation under the investigated conditions of this study [12] .

- *Shelf Life Determination*

Based on data obtained from the kinetics of degradation, reaction rate constants of Vit C (k) at the temperature of 37 and 45 °C (K_{37} and K_{45}) were calculated. Using Arrhenius equation: $K=Ae^{-Ea/RT}$ (3) and the rate constants: K_{37} and K_{45}, the activation energies (Ea), the rate constant at room temperature (K_{20}) were calculated [13]. The shelf lives of Vit C (t_{90}) were then determined. t_{90} is the time after which Vit C concentration decreases by 10 % in the solution [14].

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III. RESULTS AND DISCUSSION

➢ *Particle Size*

Microparticles were prepared by solvent- evaporation method. The prepared particles were spherical without significant deformation (for F1 and F2 when tween was used: the number of deformed particles was a little bigger than the other experiments probably because of the inability of tween to stabilize emulsion like PVA).

The size of particles ranged between 60 and 102µ (TABLE 2). Tween doesn't increase outer phase viscosity, particles sizes of F1 and F2 were smaller than that of F3.

Table 2 Particle Size and EE of Different Experiments

	Mean particle size±SD (µ)	EE%
F1	70±3.1	25±2
F2	72±4.5	26±1.3
F3	81±6.1	32±2.4
F4	69±3.2	43±1.1
F5	102±6	44±0.8
F6	60±4.2	36±2.5
F7	68±3.5	37±2.1
F8	67±3.1	29±3.4

When PVA concentration decreased (F4) and polymer quantity increased (F5), particle size decreased and increased respectively and this can be explained by decreasing or increasing viscosity respectively [15] which results in increase or reduction of stirring efficiency [16].

The high stirring in F6 provided the necessary energy for reduction particle size [6, 15] down to 60 µ.

Smaller particles were obtained in F8 when sucrose was added in outer phase, sucrose generated water flow from inner phase because of its osmotic properties [6, 17].

➢ *Encapsulation Efficiency*

The EE ranged between 25% and 44%. When tween was used instead of PVA, EE decreased (25 and 26 against 32%) in F1, F2 and F3 respectively and this can be attributed to increasing effect of PVA on viscosity [17]. Viscosity enhances emulsion stabilization and restricts probably VIT C movement toward outer phase.

Decreasing PVA concentration in F4 was accompanied by higher EE (43 against 32% in F3). This could be explained by lower viscosity in F4 (in comparison with F3) which permits easy DCM movement and consequently rapid solidification of particles.

Increasing polymer quantity (F5) led to EE increase (44% against 30% in F3) and this may be explained by enhanced viscosity which hindered VIT C transfer towards the outer phase [6, 18].

When F6 microparticles are compared to F3 particles, microparticles of F6 are small and have large surface area that probably allowed DCM transfer and resulted in rapid particles solidification. For this reason, higher EE was shown in F6.

When VIT C quantity increased (F7), higher EE was recorded.

Because of its osmotic effect [6, 17], sucrose in outer phase (F8) attracts water from inner phase carrying VIT C. Smaller EE was thus observed in F8 (29%) against 32% in F3.

➢ *Determination of Vit C Shelf Life Under Different Conditions*

TABLES 3 and 4 show R^2 values of degradation rates of Vit C in outer and inner phases at both temperatures: 37˚ and 45˚ C. VIT C follows first order degradation kinetic as reported by previous studies [19] and its degradation is concentration dependent.

Table 3 The Determination Coefficients: R^2_1 (First Order) and R^2o (Zero Order) of Vit C Degradation Kinetics at 37˚ and 45˚ C (Outer Phase)

		F1	F2	F3	F4	F5	F6	F7	F8
37˚ C	**R^2_1**	0.9868	0.9973	0.991	0.9899	0.997	0.9904	0.9936	0.99
	R^2o	0.9711	0.9877	0.99	0.9983	0.991	0.9899	0.9822	0.9843
45˚ C	**R^2_1**	0.9858	0.9979	0.9977	0.9967	0.9936	0.9828	0.9868	0.9926
	R^2o	0.9591	0.9857	0.9863	0.9897	0.9806	0.9756	0.9861	0.9879

Table 4 The Determination Coefficients: R^2_1 (First Order) and R^2o (Zero Order) of Vit C Degradation Kinetics at 37˚ and 45˚ C (VIT C Entrapted in Inner Phase)

		F1	F2	F3	F4	F5	F6	F7	F8
37˚ C	**R^2_1**	0.9965	0.9987	0.9956	0.9943	0.9912	0.9865	0.9823	0.9925
	R^2o	0.9863	0.9957	0.9746	0.9842	0.9813	0.9718	0.97	0.9878
45˚ C	**R^2_1**	0.9878	0.9796	0.9769	0.9952	0.9844	0.9878	0.9868	0.9769
	R^2o	0.9796	0.9734	0/99	0.9912	0.9959	0.9796	0.958	0.9718

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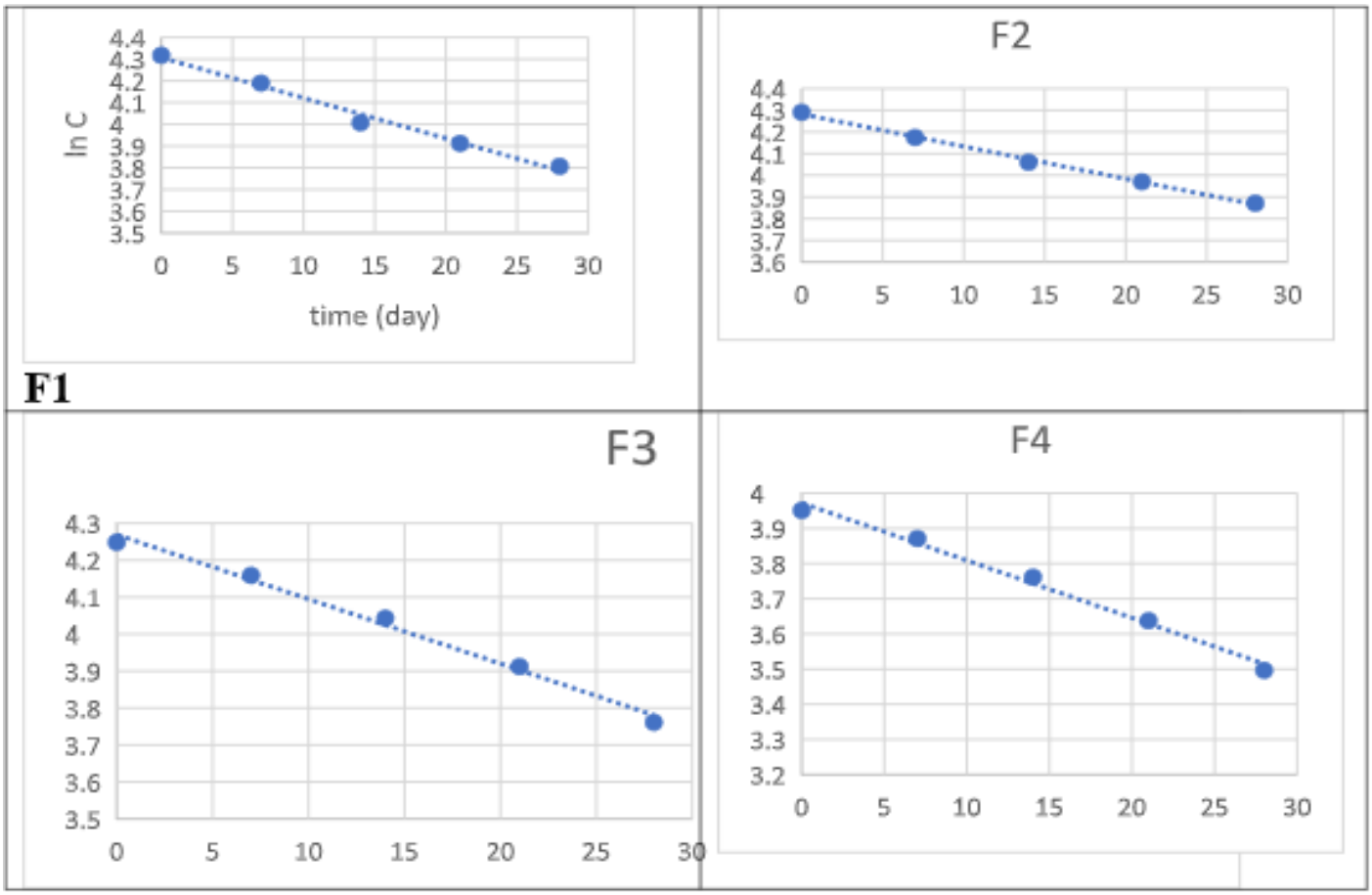

Fig 2 Illustrates the Profiles of Vit C Degradation at 37C According to First Order

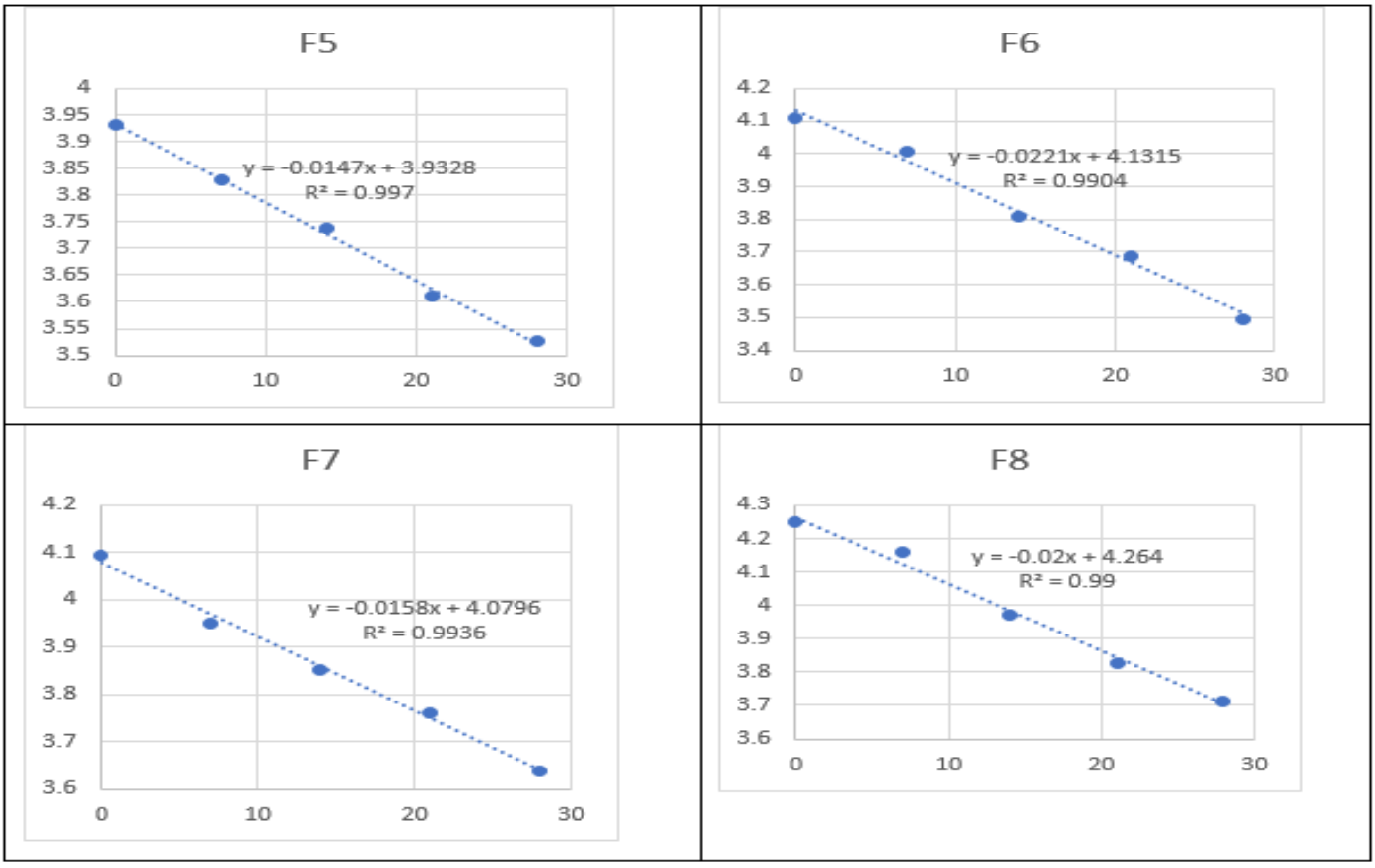

Fig 3 Profiles of Vit C Degradation at 37C According to First Order (Outer Phase)

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The calculated shelf lives of Vit C are gathered in TABLES 5 and 6. They range between 6 and 12 days (outer phase) and range between 12 and 23 in inner phase.

Under investigated conditions, emulsion stabilization by 2% tween 80 could stabilize VIT C in outer phase more than PVA could (TABLE 5). This may be due to impurities found in PVA powder during its manufacturing [17].

Increasing VIT C quantity during microencapsulation led to EE increase however the VIT C quantity in outer aqueous phase was still higher than that of other experiments (almost 0.3g). Increasing VIT C concentration is accompanied with higher stability [20] however the color of outer phase in F7 was more yellow than the reference F3 because of more degraded VIT C due to higher concentration of VIT C.

Sucrose can protect VIT C from degradation by increasing viscosity [21] and reducing water activity [22]. For this reason, VIT C in outer phase of F8 was stabilized more than VIT C in F3.

The other experiments (F4, F5, F6) have the same ingredients and conditions in outer phases as F3 so VIT C shelf lives in outer phases for these experiments were almost similar.

From TABLE 6, it is obvious that microencapsulation can protect VIT C from degradation. This finding agrees with other studies [23, 24, 25, 26, 27]. Microencapsulated VIT C is more stable rather than in solution [28].

Table 5 The Shelf Lives (t_{90}) of Vit C in Outer Phase

	F1	F2	F3	F4	F5	F6	F7	F8
t_{90} (day)	7	10	9	6	9	7	12	10.5

Table 6 The Shelf Lives (t_{90}) of Vit C Encapsulated in Inner Phase

	F1	F2	F3	F4	F5	F6	F7	F8
t_{90} (day)	19	12	12	16	23	19	21	17

IV. CONCLUSIONS

VIT C microparticles were prepared by solvent-evaporation of solvent. Particles size was affected by PVA and polymer concentrations and by speed of agitation. EE was influenced by PVA, VIT C and Eudragit concentrations. EE was also affected by speed of agitation. VIT C can be protected in aqueous solutions when sucrose and tween were added. Microencapsulation can extend VIT C shelf life in all experiments up to 23 days.

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