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Study of the Effect of Different Additives on the Shelf Life of Microencapsulated Vitamin C

Makhlouf Nour[*1]; Harfouch Judi[2]; Al Haushey Lama[3]
Faculty of Pharmacy, Al Sham University, Latakia, Syria
Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, Tishreen University, Latakia, Syria

*Corresponding Author:- Makhlouf Nour[*1]

Abstract:- Vitamin C (Vit C) is sensitive to oxidation, so maintaining its stability is the biggest challenge in its preparation and use. The aim of this research was to study the effect of adding various additives on the stability of Vit C microencapsulated in microparticles prepared by solvent evaporation method. The results showed that the system viscosity had an effect on the particle size, encapsulation efficiency EE was affected by PVA, Vit C and polymer concentrations. The additives that have shown a positive effect on EE are sucrose concentration, addition of alginate Na and chitosan. The results also showed that using (sucrose, glucose, cysteine, alginate Na and chitosan) as additives can protect AA in microparticles and increase shelf lives (AA shelf life increased significantly from 15 to 42 days by using sucrose as additive).

Keywords:- *Vit C, Shelf Life, Additives, Microencapsulation.*

I. INTRODUCTION

Vitamin C is used widely in cosmetics and pharmaceutics products because of its effective properties, it has an important role as an antioxidant. it causes an increase of absorption iron, folic acid and calcium [1]. Vitamin C helps to strengthen the immune system, and it has shown a pharmacological effect in many diseases [2]. In addition to its important role in topical formulation as an anti-aging by neutralize reactive oxygen species (ROS) resulting from exposure to solar radiation and environmental factors [3, 4]. Studies also indicate that Vit C is effective in the treatment hyperpigmentation, sunspot and melasmas [3]. However, the biggest challenge in using Vit C is to keep up its stability because it shows instability for many factors such as the oxidation [1, 2, 3, 5]. the most commonly used technology to get better stability of Vit C is microencapsulation [2] which is a process in small solid particles, liquid droplets, or gas molecules are enclosed within a layer of coating, or embedded in a homogeneous or heterogeneous matrix. This technique can protect the enclosed active ingredient from the external environment and releases [6, 7]. Many techniques can be utilized to encapsulate Vit C. Unfortunately, there is no single method can be fully effective to achieve stability. Each technique has advantages and limitations, that makes selection a method vary from case to case. The used methods to encapsulate Vit C are commonly solvent evaporation, spray drying, spray chilling, spray cooling, fluidized bed coating, liposomes and extrusion. Solvent evaporation was chosen because it is a simple and cheap method [4]. In the literature, several studies have shown a positive effect of different additives (sucrose, glucose….) on the stability of Vit C [8, 9].

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

II. MATERIAL AND METHODS

A. Material

Vit C was purchased from Loba chemie, India. 2,6-dichorophenol: Indophenol Sodium was obtained from Tmmedia, India. Eudragit RS 100, alcohol poly vinyl PVA, sucrose, glucose, cysteine and alginate Na were obtained from Sigma-Aldrich. Oxalic acid and chitosan were of analytical grade.

B. Methods

- *Microparticles Preparation*

Vit C microparticles were prepared by double emulsion solvent-evaporation according to Al haushey et al. [10] with some modifications. Briefly, an aqueous solution of Vit C was vortexed (VELP scientifica, 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) in little volume of outer aqueous containing 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. Different additives (sucrose, glucose, cysteine, alginate Na and chitosan) were added in inner aqueous phase W1.

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Table 1 Different Variables in Vit C Microparticles

	A	B	C	D	E	F	G	H
Sucrose (g)	0.1	0.25	_	_	_	_	_	_
Glucose (g)	_	_	0.1	0.25	_	_	_	_
Cysteine (g)	_	_	_	_	0.1	0.25	_	_
Alginate Na (g)	_	_	_	_	_	_	0.1	_
Chitosan (g)								

➢ *Vit C Determination*

The bioactive Vit C was determined using 2,6-dichlorophenol: indophenol (DCPIP) method according to AOAC official method (1984) [11] with minor modification in which Metaphosphoric acid was substituted by oxalic acid (0.2%). Titration methods are cheap and don't request sophisticated equipment [12]. 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) according to Sudhamani et al. [13]. The mean diameter was calculated according to the equation:

$$d= (\in nidi)/(\in ni)$$

- *Kinetic Modeling*

A zero-order model: C=C0-Kt (1) and first order model: ln C=lnC0-Kt (2) were fitted to determinate the kinetic of Vit C degradation under the investigated conditions of this study [16].

- *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 (K37 and K45) were calculated. Using Arrhenius equation (3) and the rate constants: K37 and K45, the activation energies (Ea), the rate constant at room temperature (K20) were calculated [17]. The shelf lives of Vit C (t90) were then determined. t_{90} is the time after which Vit C concentration decreases by 10 % in the solution [18].

- *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) during 15 minutes. The encapsulated Vit C was calculated according the following:

EE%= (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 [14, 15]. 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.

III. RESULTS AND DISCUSSION

➢ *Particle Size*

Microparticles were prepared by solvent- evaporation method. The prepared particles were spherical without significant deformation.

The size of particles ranged between 52 and 84 (TABLE 2). When sucrose (0.25%), alginate Na and chitosan were added (B, G and H respectively), microparticle particle size increased because of positive effect of these additives on viscosity [19].

Stirring is more efficient in systems with low viscosity [20] therefore, particles of E and F (when cysteine was used) were smaller than the others (68 and 71 microns respectively). The other factors didn't play significative role.

Table 2 Particle Size and EE of Different Experiments

	Mean particle size±SD (µ)	EE%
A	80±4.1	38±2.6
B	85±5	45±3.3
C	75±4.1	38±2.6
D	77±5.5	40±1.9
E	68±6	30±2.8
F	71±5.3	31±3.7
G	87±3.5	60±3.1
H	90±4.3	55±3.7

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➢ *Encapsulation Efficiency*

The EE ranged between 30% and 60%. When sucrose was used at 0.25%, EE increased (45 against 38%) and this can be attributed to increasing effect of sucrose on viscosity (Handbook). Viscosity enhances emulsion stabilization and hinders probably Vit C leakage in aqueous outer phase. In addition, and by creation an osmotic pressure inside the particles that attracts water leading to swelling of droplets [10]. Glucose at two investigated concentrations didn't increase EE when compared to sucrose at 0.1% because probably, there was no significative effect on viscosity or on any other factor enhancing EE.

On the other hand, cysteine has no effect on viscosity like sucrose therefore, EE in C and D (when cysteine was added at tow concentrations) was low (30 and 31%). Alginate Na and chitosan increase water viscosity and consequently Vit C movement toward outer phase restricts. High EE (s) were thus obtained for G and H (60 and 55%) in comparison with A (38%). It appeared that viscosity had very important effect on Vit C stabilization and this is found in previous studies [21, 22].

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

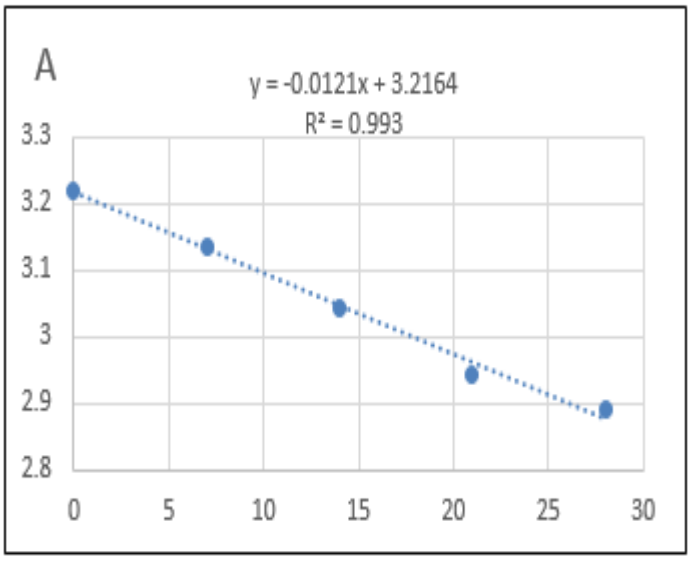
TABLES 3 and 4 show R2 values of degradation rates of Vit C in outer and inner phases at both temperatures: 37˚ and 45˚ C. Vit C follows first order degradation kinetic as reported by previous studies [23] and its degradation is concentration dependent.

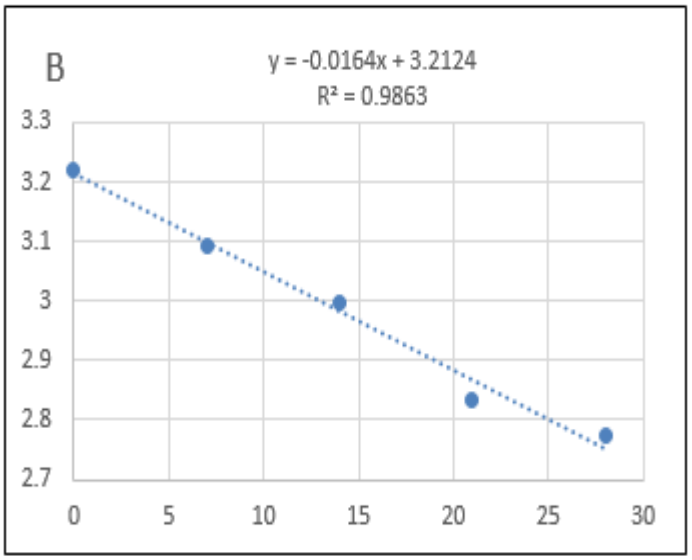
Table 3 The Determination Coefficients: R^2_1 (First Order) and R^2o (Zero Order) of Vit C Degradation Kinetics at 37˚ and 45˚ C (Free Vit C in Outer Phase)

		A	B	C	D	E	F	G	H
37˚ C	R^2_1	**0.987**	**0.9982**	**0.9912**	**0.9961**	**0.9932**	**0.9964**	**0.9962**	**0.995**
45˚ C	R^2o	**0.9751**	**0.9862**	**0.993**	**0.9832**	**0.9913**	**0.9884**	**0.9802**	**0.9872**
	R^2_1	0.9872	0.9974	0.9972	0.9987	0.9956	0.9885	0.9863	0.9976
	R^2o	0.9791	0.9877	0.9893	0.9863	0.9818	0.9765	0.9821	0.9845

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)

		A	B	C	D	E	F	G	H
37˚ C	**R^2_1**	0.993	0.9863	0.984	0.991	0.9834	0.9916	0.9889	0.9823
	R^2o	0.985	0.9956	0.978	0.9875	0.9873	0.9716	0.971	0.9868
45˚ C	**R^2_1**	0.988	0.9787	0.989	0.9962	0.9943	0.9861	0.9865	0.9779
	R^2o	0.9737	0.9734	0/9889	0.9918	0.9986	0.9786	0.9582	0.9728





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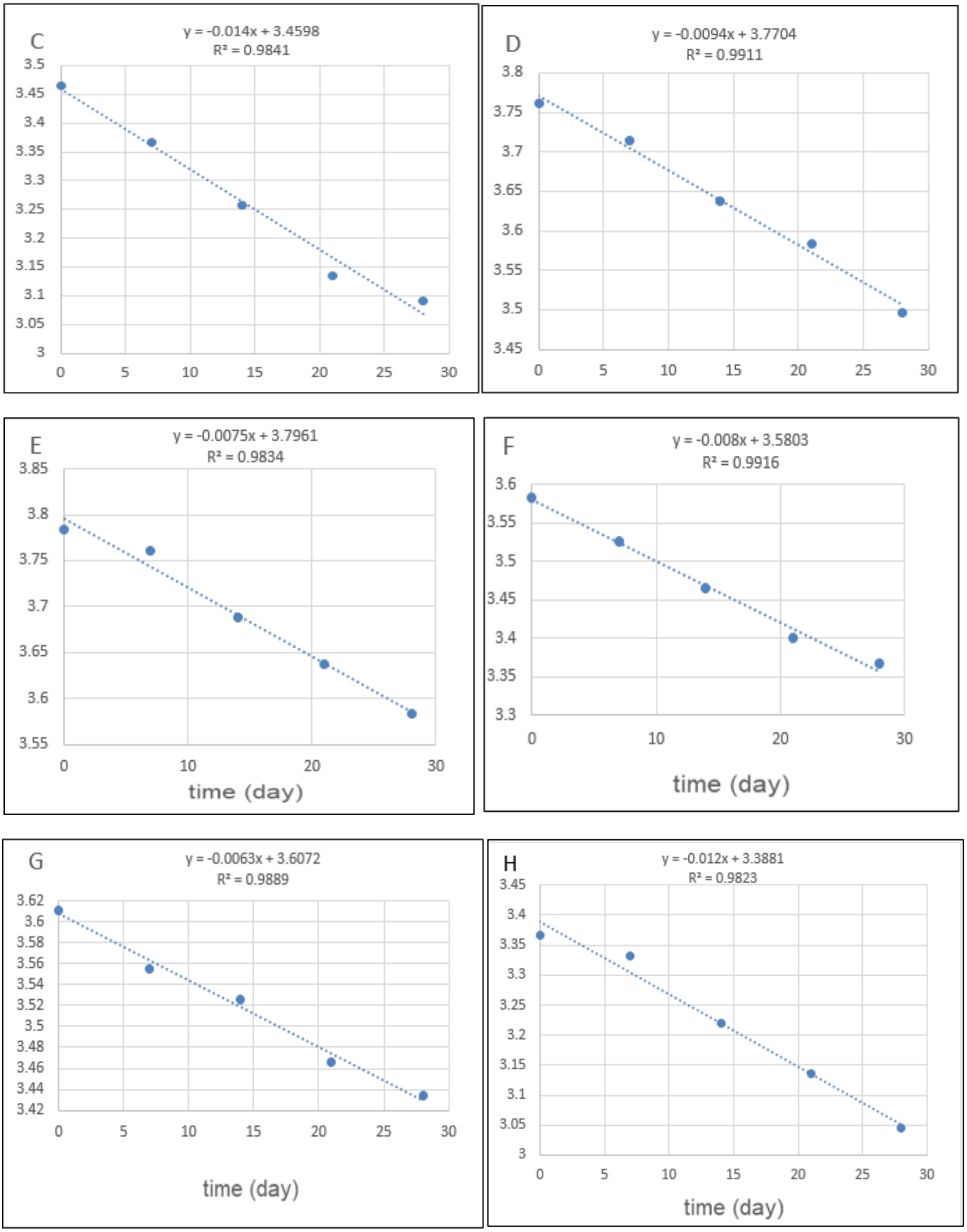

Fig 1 Profiles of Encapsulated Vit C Degradation at 37C According to First Order

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The calculated shelf lives of Vit C are gathered in TABLES 5 and 6. They range between 12 and 23 days in outer phase and between 15 and 42 in inner phase.

From TABLE 6, it is obvious that microencapsulation can protect Vit C from degradation (shelf lives of encapsulated Vit C were higher than those in aqueous outer phases). This finding agrees with other studies [24, 25, 26, 27, 28]. Microencapsulated Vit C is more stable rather than in solution [29]

Unless the additives were added in inner phase, only the results concerning the shelf life of encapsulated Vit C will be analyzed.

Sucrose is known to stabilize Vit C because it enhances viscosity [8] and reduces water activity [30]. Therefore, Vit C shelf life increased significantly from 15 to 42 days (A and B).

Glucose also enhances Vit C stability [31]. Glucose could stabilize Vit C more than sucrose (15 for sucrose against 22 for glucose) and this could be attributed probably to reducing capacity of glucose which helps scavering oxidizing agent more rapidly than Vit C. However, sucrose at 0.25% was more effective in keeping Vit C stability maybe because its ability to bind with water more than glucose. In the work of Palanisamy et al., sucrose in its eutectic mixture can bind water by hydrogen bonds more than glucose which result in more Vit C stabilization [32].

Cysteine works as antioxidant [33] and can reserve Vit C integrity more than sucrose at 0.1%. it appeared that sucrose at 0.1% wasn't very effective in maintaining Vit C stability.

For alginate Na and chitosan, the shelf lives were 40 and 38 days respectively. Both additives are able to stabilize Vit C [34, 35] more than other investigated additives in this study. These results were obtained probably because alginate Na and chitosan increase water viscosity and decrease water activity by binding water molecules.

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

	A	B	C	D	E	F	G	H
t_{90} (day)	**12**	**23**	**16**	**17**	**14**	**15**	**22**	**20**

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

	A	B	C	D	E	F	G	H
t_{90} (day)	**15**	**42**	**22**	**24**	**18**	**19**	**40**	**38**

IV. CONCLUSION

Vit C microparticles were prepared by solvent-evaporation of solvent. Particles size was affected by system viscosity. EE was influenced by PVA, Vit C and Eudragit concentrations. EE was enhanced by sucrose concentration and by alginate Na and chitosan. Vit C can be protected in microparticles and shelf lives increased when different additives were used (sucrose, glucose, cysteine, alginate Na and chitosan).

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