

## FORMULATION AND EVALUATION OF SUSTAINED RELEASE TABLETS CONTAINING ANTIOXIDANTS

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إن نقص مضادات الأكسدة- يلعب دوراً هاماً في أمراض نقص المناعة كما هو الحال في مرض الإيدز وهناك كثير من مضادات الأكسدة مثل فيتامين أ ، وفيتامين ج وفيتامين د – وعنصر الزنك – والسيلينيوم – في السوق على هيئة كبسولات ولكن هذه الكبسولات مصممة على أن تكون سرعة إنسياب هذه المواد كبيرة وبالتالي تكون سرعة الأيض كبيرة وبذلك يخلو الجسم منها في فترة وجيزة. لذا كان من الضروري عمل دراسة بحثية لصياغة هذه المواد في شكل أقراص ممتدة المفعول يكون لها دور فعال في علاج مرض الإيدز لذا أستخدمت الدراسة في هذا البحث صياغة وتقييم أقراص ممتدة المفعول على المستوى المعمل والصناعي لهذه الأقراص التي تحتوي على البيتاكاروتين ، فيتامين ج ، توكوفيرول ، سيلينيوم ، الخميرة ، وبيدولات الزنك وقد تم اختيار فيتامين ج كمؤشر لتقييم الأقراص المحضرة ممتدة المفعول بالإضافة الى دراسة درجة الثبات الكيميائي لهذا العقار في محلول 0.1 عيارى من حمض الأيدروكلوريك (أس أيدروجيني 1.2) خلال 8 ساعات عند درجة حرارة 37°م. وقد تمت دراسة توافق محتوى الأقراص المحضرة مع النصوص الدستورية للأقراص ممتدة المفعول بالإضافة الى دراسة معدل إنطلاق العقار خلال 8 ساعات عند درجة حرارة 37°م.

*Deficiency in nutrients especially antioxidants play an important role in the pathogenesis of acquired immuno-deficiency syndrome (AIDS). Antioxidants; vitamin A, vitamin C, vitamin E, zinc and selenium are available in market as capsules. The release rate is very fast followed by fast elimination. It is necessary to prolong the residence time of antioxidants. Sustained release tablet formulation was designed and evaluated on small scale and on large scale preparations. Low attention has been discuss the use of sustained release formula in treatment of AIDS. Sustained release tablet formulation was designed and evaluated on small scale and on large scale preparations. The formulated tablets contained beta-carotene, vitamin C, tocopherol acetate, selenium, yeast and zinc pidolate. Vitamin C was chosen as tracer for detection and evaluation of tablet dosage form. Vitamin C was found to be stable in 0.1 N HCl (pH 1.2) along 8 hrs at 37°C. The formulated tablets passed the pharmacopeial requirements for all tablet tests. The formulated tablets showed sustained release characteristics of vitamin C as a tracer, within 8 hrs.*

### INTRODUCTION

Immediate release dosage forms result in a quick rise of blood plasma levels with a subsequent decrease due to elimination. The use of sustained release medication could be beneficial in order to maintain therapeutic plasma levels. Furthermore, it would improve the patient compliance with the use of once daily drug administration<sup>1</sup>. Although initial development costs may be high, controlled release dosage forms have the potential to

enhance clinical efficacy and reduce the total treatment cost as compared to immediate release dosage forms<sup>2</sup>.

Since the beginning of the epidemic of AIDS, researchers provided the obviousness, at what stage, AIDS could be prevented, treated and be controlled by guaranteeing an optimal nutritional state to the patients<sup>3</sup>. Actually, one can mention that optimal nutrition can help boost immune function, maximize the effectiveness of antiretroviral therapy, reduce the risk of opportunistic infections and chronic

illnesses such as diabetes and cardiovascular diseases, and contribute to a better overall quality of life<sup>4</sup>. Malnutrition, weight loss, and body cell mass (BCM) depletion have long been observed in individuals with HIV infection and acquired immunodeficiency syndrome and are associated with increased morbidity and mortality<sup>5</sup>. Nutritional deficiency play a significant role in the immunodeficient state. It was also reported that the severity of clinical manifestations of AIDS is proportional to the degree of nutritional deficiency<sup>6&7</sup>.

Since the beginning of the epidemic of AIDS, the free radicals and specifically the antioxidant agents were implied in the pathogenesis of this new syndrome<sup>8-10</sup>. The role of the oxygen free radicals in the HIV/AIDS was the subject of many congresses<sup>11&12</sup>. The nutritional therapy proposed in AIDS, was discussed<sup>13&14</sup>, used at the beginning of the epidemic and still used currently. Studies consistently show that serum antioxidant vitamins and minerals decrease while oxidative stress increases during AIDS progression. The optimization of nutritional status, intervention with foods and supplements, including nutrients and other bio-active food components, are needed to maintain the immune system. Various food components may be recommended to reduce the incidence and severity of infectious illnesses by forms of bio-protection which include reduced oxidative stress due to reactive oxygen species which stimulate HIV replication and AIDS progression<sup>15</sup>.

Antioxidants are available in the market as capsules. Their release rate is very fast. This leads to depletion of antioxidants from the body within short time. To raise the efficiency of antioxidant system in patients, it is recommended to prolong the residence time of antioxidants. Therefore, the aim of this work is to study the effect of formulation factors on the stability of vitamin C as a tracer for formulae selected. The second step is to formulate and evaluate sustained release tablets containing antioxidants. This specific sustained release formula has never been tested in AIDS before<sup>16</sup>. The research will be extended to be applied on human HIV positive volunteers after approval of research ethic's committee.

## MATERIALS AND METHODS

### Materials

All materials are of pharmaceutical grades were bought from DistriB3 Company (Marseille).

- Betacarotene 10 CWS (10%) (Vitamin A),
- Ascorbic acid coated with ethylcellulose (97.5%) (Vitamin C),
- Tocopherol acetate 50 CWS (50%) (Vitamin E),
- Selenium yeast (0.2%),
- Zinc pidolate (20%).

Other materials are listed in Table I.

### Methods

#### Tracer selection

Different active principles are present in the formula under investigation: vitamin A, vitamin C, vitamin E, in addition to selenium and zinc. All these materials are of diverse physicochemical nature. For this reason vitamin C was chosen as a tracer for detection and evaluation of other active principles. Vitamin C was coated with ethylcellulose in order to increase its stability. The dose of vitamin C in each tablet was 110 mg. The wavelength of vitamin C in UV was determined in different pH values 1.2, 4.5 and 6.8. The stability of vitamin C at different pH values was studied.

#### Pharmaceutical formulation

##### Small scale formulation

Table II illustrates 16 formula suggested for tablet formulations. All tablets were evaluated according to the requirement of European Pharmacopoeia 2008<sup>17</sup>.

Direct compression technique (Frogerais alternative single punch type OA) was used for tablet preparation. Table III illustrates the constituents of tablets according to daily requirement.

Vitamins A and E are oily in nature, so it is recommended to use adsorption technique, for preparation of tables. Vitamin A and E were adsorbed on Aerosil® 200 and Lactose respectively to facilitate mixing with other materials. The final formula contains 50/50 active component / excipients.

##### Large scale formulations

Trials were made to apply the recommended formula (Table III) in a large

**Table I:** List of excipients used in tablet preparations.

Class	Trade name	Chemical name
Binders	Kollidon® K 30	Homopolymere 1-Ethenyl-2-pyrrolidinone
	Kollidon® VA 64	Polyvinylpyrrolidone
	CMC sodium	Sodium carboxymethylcellulose
Fillers	Lactose SD monohydrate	O-D-Galactopyranosyl-(1(4)- $\alpha$ -glucopyranose (anhydrous or monohydrate)
	Avicel® PH 102	Microcrystalline cellulose
	Dibasic calcium phosphate	Dibasic calcium phosphate dehydrate
Lubricants	Aerosil® 200	Sodium dioxide
	Magnesium Stearate	Magnesium stearate
	Talc	Magnesium silicate
	Sodium stearyl fumarate	Sodium stearyl fumarate
Sustained release excipients	Precirol® ATO 5	Glycerol stearyl palmitate
	Compritol® 888 ATO	Glycerol tribehnate
	Eudragit® RS PO	Ethylacrylate (copolymer)
	Metolose 90 SH 400/1500/100 000	Methylcellulose
	EC powder	Ethylcellulose

**Table II:** Tablets ingredients.

Mix	Binders	Fillers	Lubricants	Sustained release excipients
1	Kollidon K30	Lactose SD monohydrate	Aerosil 200	Precirol ATO5
2	Kollidon K30	Avicel PH 102	Sodium Stearyl fumarate	Metolose 90 SH400
3	Kollidon VA64	Lactose SD monohydrate	Talc	Compritol 888 ATO
4	Kollidon VA64	Dibasic calcium phosphate	Sodium Stearyl fumarate	Precirol ATO5
5	Kollidon VA64	Lactose SD monohydrate	Aerosil 200	Metolose 90 SH100000
6	Kollidon VA64	Dibasic calcium phosphate	Magnesium Stearate	Metolose 90 SH1500
7	Kollidon VA64	Avicel PH 102	Talc	Eudragit RS PO
8	CMC sodium	Avicel PH 102	Talc	Precirol ATO5
9	CMC sodium	Lactose SD monohydrate	Magnesium Stearate	Metolose 90 SH100000
10	CMC sodium	Avicel PH 102	Aerosil 200	Metolose 90 SH1500
11	CMC sodium	Lactose SD monohydrate	Talc	Ethylcellulose
12	CMC sodium	Dibasic calcium phosphate	Sodium Stearyl fumarate	Eudragit RS PO
13	Kollidon K30	Dibasic calcium phosphate	Aerosil 200	Compritol 888 ATO
14	Kollidon K30	Dibasic calcium phosphate	Talc	Metolose 90 SH400
15	Kollidon K30	Lactose SD monohydrate	Sodium Stearyl fumarate	Metolose 90 SH1500
16	Kollidon K30	Avicel PH 102	Magnesium Stearate	Ethylcellulose

**Table III:** Tablet formulation according to daily requirement.

Materials	Weight in each tablet (mg)	Weight for 100 tablets (g)
1- Active components		
Vitamin A	50.000	5.000
Vitamin C	112.820	11.282
Vitamin E	24.000	2.400
Zinc	60.000	6.000
Selenium	50.000	5.000
2- Excipients		
- Kollidon K30	29.68	2.96
- Dibasic calcium phosphate	178.09	17.81
- Talc	29.07	2.97
- Metolose 90 SH400	59.36	5.94

scale, using 1.187 kg to produce 2000 tablets. Frogerais PR16 Rotary tablet press was used (punch of 11 mm). Talc was selected as a lubricant for its cheap price, and being not sticky to the machine.

#### Tablets evaluation

Hardness test, stability of vitamin C in tablets, disintegration test, content uniformity and friability tests were performed for all tablets defined in the experimental part.

#### Hardness test

Tablets were evaluated using hardness tester (TBH 200D, Erweka GmbH, Germany).

#### Stability of vitamin C in granules

Uncoated and coated vitamin C granules, were pulverized separately in a mortar and dissolved in one liter aqueous media pH 1.2. The contents were measured during 8 hrs at room temperature and at 37°C, using UV spectrophotometer (Shimadzu-150-02 Seisakusho Ltd., Tokyo, Japan), at wavelength 243.4 nm. The variations between tablets were calculated.

#### Disintegration test

Disintegration test was performed according to European Pharmacopoeia<sup>17</sup>. The test was performed on six tablets (Erweka tablet disintegration apparatus GmbH, Germany).

#### Content uniformity

One tablet was powdered in a mortar, and dissolved in one liter 0.1 N HCl (pH 1.2). The test was repeated ten times. The contents of each tablet of vitamin C were determined using UV spectrophotometer at wavelength  $\lambda = 243.4$  nm.

#### Friability test

The friability test was performed using Erweka tester (Germany). The apparatus was adjusted at 25 rounds/minute during 4 minutes<sup>17</sup>. The test was performed on 10 tablets, and repeated three times, and the average was calculated.

#### Dissolution test

The dissolution test<sup>17</sup> was performed on two optima formulae selected by previous tests<sup>18</sup>. The test was performed using dissolution apparatus (SOTAX DT3 German). Six tablets for each optimum lot were used for dissolution test, using paddle method (50 rpm). Dissolution media was one liter 0.1 N HCl (pH 1.2), at 37°C during 8 hrs.

## RESULTS AND DISCUSSION

#### Small scale formulations

##### Tracer evaluation

Vitamin C was used as a tracer for tablets evaluation. The results indicated that no interference was seen between ingredients

present in the formula with vitamin C evaluation at pH 1.2.

Maximum absorbance of vitamin C was found to be 243.4 nm, 256 nm and 265.4 nm in pH 1.2, pH 4.5 and pH 6.8 respectively. A linear correlation between absorbance and concentration of vitamin C (pH 1.2) was found (up to 132 mg/L).

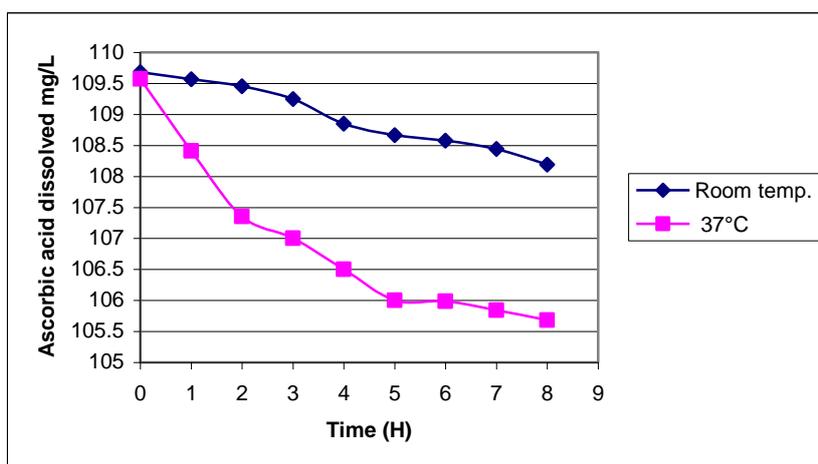
### Stability of vitamin C

The stability of uncoated vitamin C tablets in different pH 1.2, 4.5 and 6.8 at room temperature was studied. The results revealed that vitamin C is most stable at pH 1.2 compared with the media of pH 4.5 and pH 6.8.

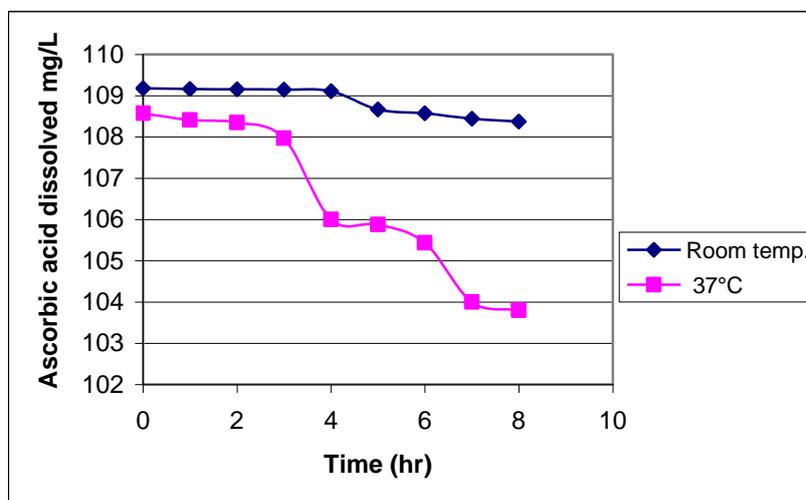
Therefore, the media of pH 1.2 was selected for testing the sustained release behavior for tablets.

On the other hand, the stability of vitamin C granules coated with ethylcellulose was studied. The granules were dissolved in HCl (pH 1.2). The stability was performed at room temperature and at 37°C. It is obvious that the loss of vitamin C during 8 hrs attained 1.35% at ambient temperature and 3.55% at 37°C for uncoated sample (Fig. 1).

The percent loss of vitamin C coated with ethylcellulose at pH 1.2 during 8 hrs attained 0.68% at ambient temperature and 2.50% at 37°C (Fig. 2).



**Fig. 1:** Stability of uncoated ascorbic acid granules in media of pH (1.2) at different temperatures.



**Fig. 2:** Stability of coated granules with ethylcellulose using a media of pH (1.2) at different temperatures.

## Tablets formulation

Table IV illustrates different parameters for different combinations of tablet excipients namely; apparent volume (V<sub>10</sub>-V<sub>500</sub>), flowability, weight variations, hardness, disintegration time and amount dissolved after 8 hrs from tablets. All data recorded obey the pharmacopeial requirements<sup>17</sup>.

Formula 2 and 14 were selected according to the best friability and hardness requirements (Table IV). The dissolution behavior of vitamin C from tablets depends mainly on the behavior of excipients in dissolution medium. Consequently, the hydrophilic excipients, e.g. lactose is responsible for rapid liberation of drug. On the other hand, on using Aerosil (F10) (Table II), the dissolution rate decreased.

The same results were obtained on using Compritol, Precirol and dibasic calcium phosphate as filler (F3, F4, and F14), and also on using CMC sodium with Avicel (F8). Moreover, CMC sodium mixed with Avicel, improved the compressibility of tablets. This hypothesis, could be applied on F10, F13 and F14 which contain Metelose/CMC, Kollidon/ Compritol and Kollidon/methylcellulose respectively.

It is worthy to note that when two viscous materials with soluble or insoluble fillers, may increase or decrease the dissolution behavior of the active principles respectively.

According to aforementioned discussion; the two formula of choice are F2 and F14 which gave the recommended behavior (Table IV and Fig. 3). The two formula selected differ only on filler and lubricant used. Talc was chosen (F14) because it is the cheapest.

## Tablets evaluation

### Flowability test

The flowability of the tablet formulae in powder form (100 g) was 5.8 seconds for formula F2 and 5 seconds for formula F14, suggesting better weight uniformity for formula F14<sup>17</sup>.

### Apparent volume of powder

Using 100 g powder, the initial volume was measured (V<sub>0</sub>). After using 10 and 490 vibrations, V<sub>10</sub> and V<sub>500</sub> were recorded respectively. The results are shown in Table V. This value is within the limits of recommended value<sup>17</sup>.

## Uniformity of weight and hardness

Theoretical weight was 0.593 g. The mean weight (Table IV) for 10 tablets of Formula F2 was 0.594 g; the variation percent was 1.85%. The mean weight for 10 tablets of Formula F14 was 0.595 g; the variation percent was 1.06%. This is within pharmacopeial limits<sup>17</sup>. Concerning hardness test (kg/ton), the mean for 10 tablets equals 4.57 for formula F2 and 4.65 for formula F14.

## Uniformity of content

The results obtained were within the pharmacopeial limits<sup>17</sup>. The mean content uniformity is 110 mg ± 3% for formula F2 and 110mg ± 2.5% for formula F14.

## Friability test

The mean weight of 20 tablets of formula F2 before test, was P<sub>0</sub>= 11.830 g, and the mean weight after test was P= 11.724 g. The mean weight of 20 tablets of formula F14 before test, was P<sub>0</sub>= 11.768 g, and the mean weight after test was P= 11.694 g. This confirms the requirement for tablets: loss was less than 1% for both formulae (Table IV).

## Dissolution test

Six tablets from two patches (F2 & F14) were used for dissolution test. One of which contained sodium stearyl fumarate (Fig. 4) and the other contained talc as a lubricant (Fig. 5). Figures (4 and 5) show the dissolution rate of six tablets. The results revealed that a sustained release requirement was attained along 8 hrs. The drug delivery system exhibits sustained release and succeeded in delivering a total cumulative ascorbic acid quantity of 76.3% (SD 21.27) of the initial theoretical amount after 8 hrs for formula F2 (Fig. 4) and 79.9% for formula F14 (SD 22.2) (Fig. 5).

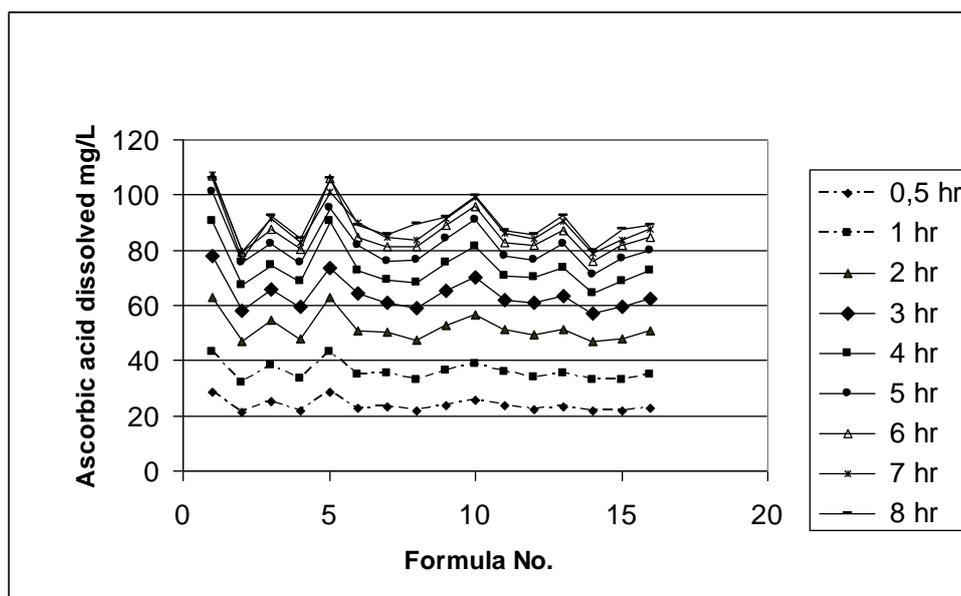
## Large scale tablet formulations

Talc was selected as a lubricant, for its cheap price, and being not sticky to the machine. So, Formula F14 mentioned in Table VI was used to prepare 2000 tablets. All quality control tests were applied to the mixed ingredients in powder form. The results of apparent volume were found to be:

$$\begin{aligned} \text{Initial volume } V_0 &= 242 \text{ ml} \\ V_{10} &= 237 \text{ ml} \\ V_{500} &= 219 \text{ ml} \\ V_{10} - V_{500} &= 18 \text{ ml.} \end{aligned}$$

**Table IV:** Pharmaceutical evaluation of excipients and tablets using different formulation.

Formula No.	V10-V500 (ml)	Flowability (100 g/sec.)	Mean weight $\pm$ SD	Hardness (kg/ton)	Friability	Disintegration time (Min)	Dissolution after 8 hrs (mg/L)
1	21	8	564 $\pm$ 2.4	2.12	1.2	25	105.9%
2	18	5.8	594 $\pm$ 1.85	4.57	<1	>30	76.3%
3	22	10	592 $\pm$ 2.6	3.1	>1	>30	92.6%
4	23	8	580 $\pm$ 2.7	4	<1	>30	84.3%
5	22	11	585 $\pm$ 2.9	3.2	<1	23	102.8%
6	22	11	582 $\pm$ 2.7	3.6	<1	30	90.9%
7	20	8	568 $\pm$ 2.4	3.9	<1	30	85.5%
8	19	9	582 $\pm$ 2.2	3.6	<1	30	89.3%
9	20	12	578 $\pm$ 2.0	4	<1	28	92.1%
10	22	11	582 $\pm$ 2.1	3.6	1.1	24	99.5%
11	21	10	582 $\pm$ 2.1	3.2	1	>30	87.3%
12	22	12	586 $\pm$ 2.2	3.6	1.1	>30	85.7%
13	23	13	560 $\pm$ 2.4	4.3	<1	>30	92.6%
14	18	5	595 $\pm$ 1.06	4.65	<1	>30	79.9%
15	21	8	572 $\pm$ 2.4	3.8	1.1	>30	87.7%
16	21	11	585 $\pm$ 2.2	3.9	1	>30	89.1%



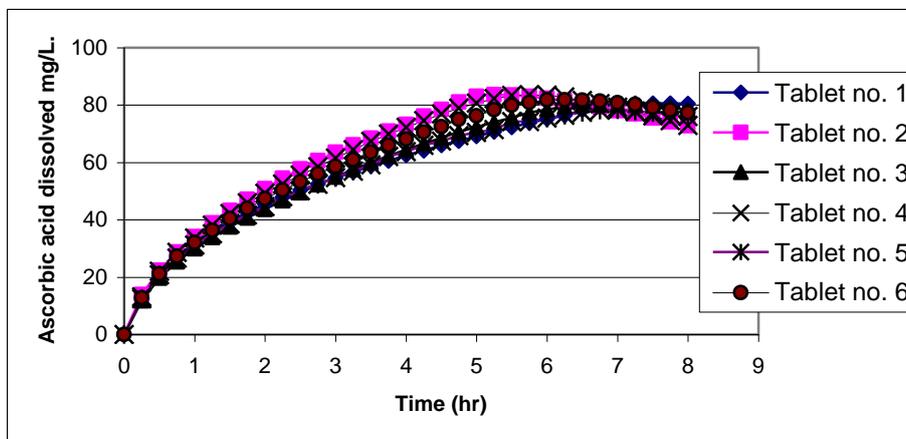
**Fig. 3:** Ascorbic acid dissolved from each formula selected.

**Table V:** Apparent volume of powder for both formulae.

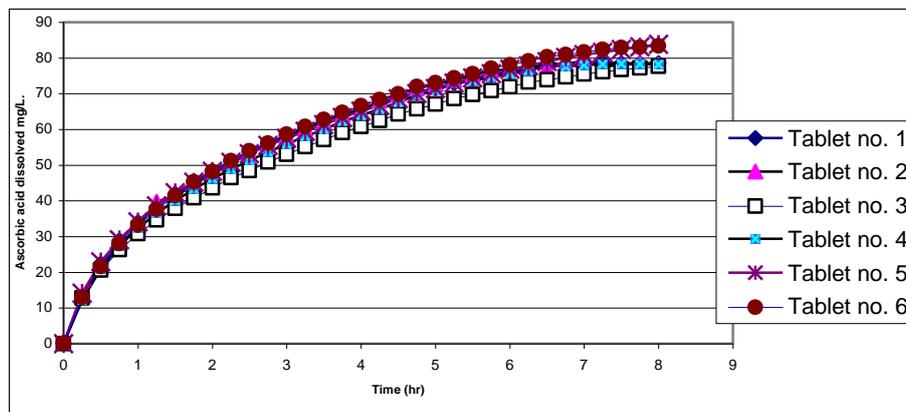
	Formula F2	Formula F14
$V_o$	245 mL	240 mL
$V_{10}$	241 mL	235 mL
$V_{500}$	223 mL	218 mL
$V_{10} - V_{500}$	18 mL	17 mL

**Table VI:** Ingredients for the two formula selected.

	Formula F2	Formula F14
Antioxidants	Vitamin A, C, E, zinc, selenium	Vitamin A, C, E, zinc, selenium
Binders	KOLLIDON K30	KOLLIDON K30
Fillers	Avicel PH 102	Dibasic calcium phosphate
Lubricants	Sodium Stearyl fumarate	Talc
Sustained release excipients	Metolose 90 SH400	Metolose 90 SH400



**Fig. 4:** Dissolution profile of 6 tablets using sodium stearyl fumarate as a lubricant and dissolution media of pH 1.2 at 37°C.



**Fig. 5:** Dissolution profile of 6 tablets using talc as a lubricant and dissolution media of pH 1.2 at 37°C.

A sample was taken from tablets and subjected to the following tests.

#### Uniformity of weight and hardness

The mean weight of 10 tablets was 0.609 g, the % variation 4.4%. This confirms the pharmacopoeial limits<sup>17</sup>. The mean hardness was  $4.04 \pm 0.6$  kg/ton.

#### Friability test

About 5 mg loss represents less than 0.8%.

#### Disintegration time

Disintegration time was found to be more than 30 min.

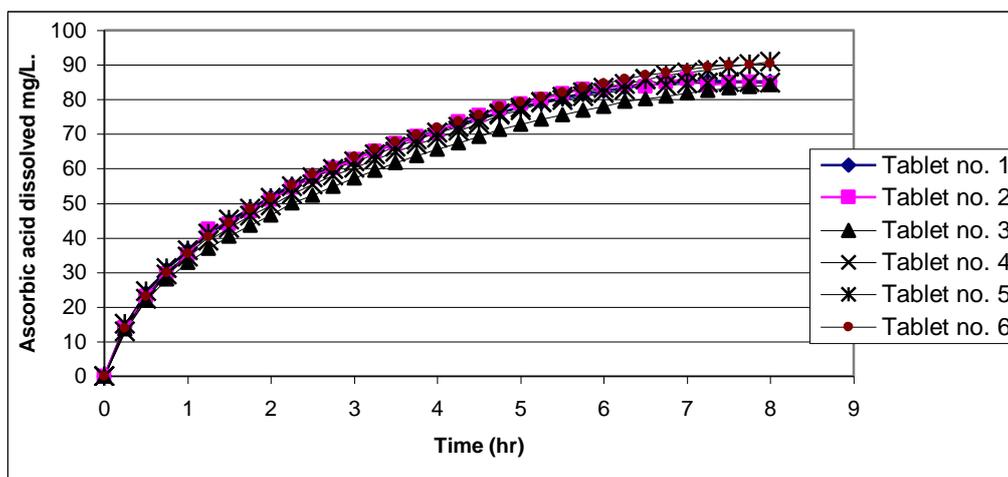
All the aforementioned results fulfill the requirements for sustained release tablet formulations<sup>17</sup>.

#### Dissolution rate

Figure 6 illustrates the dissolution profile of six tablets, from scale up patch. The results revealed that a sustained release requirement of vitamin C was attained along 8 hrs. Moreover, the percent cumulative as ascorbic acid was found to be 86.7% (SD 23.2) after 8 hrs.

#### Conclusion

In the present study, it is possible to verify the aim from this research. Formulation of a sustained release dosage form was performed to prolong the residence time of antioxidants (8 hrs) using vitamin C as a tracer. The research will be extended to evaluate the recommended formulae in human volunteers after approval of research ethic's committee.



**Fig. 6:** Dissolution profile of 6 tablets as a sample from large scale formulation using talc as a lubricant and dissolution media of pH 1.2 at 37°C.

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