Available online on 15.09.2023 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

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Research Article

Formulation and Optimization of Matrix Tablets Based on Spirulina and Vitamin C

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Article Info:

Article History:

Received 04 July 2023
Reviewed 07 Aug 2023
Accepted 23 Aug 2023
Published 15 Sep 2023

Cite this article as:

Assanhou AG, Agbokponto JE, Ganfon H, Kassehin UC, Gbessinon M, Ahyi V Yemoa LYA, Gbaguidi FA, Formulation and Optimization of Matrix Tablets Based on Spirulina and Vitamin C, Journal of Drug Delivery and Therapeutics. 2023; 13(9):41-48

DOI: http://dx.doi.org/10.22270/jddt.v13i9.6202

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Abstract

Spirulina is a type of algae widely consumed around the world as a dietary supplement due to its great nutritional potential. However, it does not contain vitamin C, a vital vitamin for the proper functioning of the human body, particularly given its immunostimulant potential. The objective of this work was to formulate sustained release spirulina–vitamin C matrix tablets that would enhance particularly a prolonged release and better absorption of vitamin C. A galenic formula based only on vitamin C was made, and then the proportion of vitamin C was reduced in favor of spirulina powder. The manufactured tablets were then subjected to various pharmacopeial quality control tests. The results of these tests showed a good distribution of the powder mixtures in the formulated tablets (i.e., mass uniformity test) and satisfactory outcomes were found for the content uniformity, disintegration and dissolution tests (with 45 % of vitamin C dissolved after 4 hours). Only the results for the friability test were unsatisfactory, indicating the need to improve the physical properties of the powders before compression. These findings open a new area for developing supplementary dietary.

Keywords: Spirulina, Vitamin C, Matrix tablet, Quality control.

INTRODUCTION

A healthy diet helps meet a person's macronutrient (protein, fat and carbohydrate, including dietary fibre) and essential micronutrient (vitamin and mineral) needs depending on gender, age, health status, physical activity and physiological state of the person [1]. According to the WHO, a healthy diet protects one against non-communicable diseases such as diabetes, heart diseases, stroke and cancer [1]. Modern cuisine, increasingly oriented towards speed and flavour, is spreading and being widely adopted throughout the world with its significant consequences on the nutritional quality of food. New kitchens use different production and processing technologies that unfortunately alter the composition of foods. As a result, the foods supposed to provide a certain number of essentials nutrients for good healthy, are found to be limited and are therefore nutritionally deficient. Since humans cannot live without an adequate level of certain exogenous nutrients, we are forced to fulfil this need through dietary supplements. Therefore, food supplements in the form of extracts and dry powders are increasingly popular with consumers and alternative medicine based on natural products (green attitude) has gained considerable recognition in the world today [2]. Nutritional supplements products represent a group of products intended to supplement a normal diet [3]. They come in various forms and fulfil different physiological roles depending on the nature of their composition. The manufacture of food supplements which arguably might not be a first investigate products as pharmaceuticals products has gradually increased it investment globally nowadays. During the Covid-19 pandemic the consumption of foods and food supplements that could boost the immune system was a widely accepted practice according to the report of the France national union of food supplements [4]. Spirulina (*Spirulina platensis*) is a cyanobacterium recognized for its high nutritional potential due to its rich composition in different nutrients (proteins,

carbohydrates, lipids), minerals (iron, calcium zinc etc.) and vitamins (A, B, E etc.) [5]. It is very rich in protein and is therefore indicated for malnourished patients. It is widely consumed around the world because it is considered as a super food supplement [6]. However, despite being a source of vital nutrients, spirulina contains little to practically no vitamin C [5]. Vitamin C is known for its; immunostimulatory effect [7]. significance in collagen synthesis [8], and facilitation of iron absorption [9]. Vitamin C is not synthesized in the body and is therefore obtained exclusively from exogenous sources (e.g., fruits, vegetables, etc) [8]. The improvement of natural immunostimulant food supplements, in order to obtain products that can increase the immune system more effectively, is the objective of this work. According to Maughan R *et al*., (2018) it is allowed to add certain substances to food supplements for the purpose of enriching them [3]. In addition, we have reported the use of Spirulina and moringa for the treatment of Corona virus as immune system booster [10]

In this study, we aimed to formulate a matrix tablet based on spirulina and vitamin C in order to improve spirulina pharmaceutical forms currently available on the market.

MATERIALS AND METHODS

1. Material

Reference ascorbic acid was obtained from the VWR/Belgium laboratory (batch number 19C114116). Powdered spirulina was obtained from local producers. The Carbopol was purchased from Hefei TMJ chemical industry (China). Vitamin C, Hydroxypropylmethylcellulose (HPMC), hydroxyl ethylcellulose (HEC) and methylcellulose (MC) were purchased from Sigma Aldrich (Belgium Pegasuslaan 5 Diegem). Avicel was obtained from cooper (Place Lucien Auvert - 77000 MELUN).

2. Pre-formulation and Formulation

Formulation with matrix agents (HPMC, HEC and MC)

The Hydroxy propyl methyl cellulose (HPMC), hydroxyl ethyl cellulose (HEC) and methylcellulose (MC) were the three matrix agents used to make vitamin C tablets by using alternatively two differents binders (Avicel and Carbopol).

The resulting tablet formulations are summarized in Table 1.

Table 1: Formulas obtained with matrix agents and binders.

	Formula A1	Formula A2	Formula A3	Formula C1	Formula C2	Formula C3
			Proportions (%)			
Vitamin C	32.7	32.7	32.7	32.7	32.7	32.7
Starch	5.8	5.8	5.8	5.8	5.8	5.8
Avicel	14.5	14.5	14.5	-	-	-
Carbopol	-	-	-	14.5	14.5	14.5
Lactose	17.3	17.3	17.3	17.3	17.3	17.3
Mg Stéarate	1.1	1.1	1.1	1.1	1.1	1.1
Talc	1.1	1.1	1.1	1.1	1.1	1.1
HPMC	27.2	-	-	27.2	-	-
HEC	-	27.2	-	-	27.2	-
MC	-	-	27.2	-	-	27.2

Formulation with spirulina

Spirulina was gradually incorporated in the formulation by varying its content (from 20 to 80%) and optimizing the various conditions to obtain the best outcome.

3. Pharmacopeial tests

The formulas which gave satisfactory tablets from the macroscopic point of view are those which were subjected to the pharmacopeial tests with specific reference to the specifications of the European Pharmacopeia 4 [11].

Friability test

The tablets (equivalent to 6 g) were dedusted then weighed before placed in the drum of the friability tester. The drum was set at 100 rotations (25 rotations per min for 4 min). The tablets were then removed and again dedusted and weighed. Finally, the lost mass was calculated by taking the difference between the initial total mass and the final total mass.

Disintegration test

The disintegration test was carried out on six (6) randomly selected tablets. The tablets were placed individually in holding tubes with a grid underneath and a disc added to each of the six tablets. The behaviour of the tablets until disintegration was followed for several hours after launching the test. The disintegration medium was milliQ water.

Mass uniformity test

Twenty (20) tablets were randomly selected from the manufactured tablets and individually weighed. The mean weight and the deviations from the mean weight were calculated.

Content uniformity test

This was carried out on ten tablets randomly chosen from vitamin C tablets and vitamin C + spirulina tablets. The ten tablets of each formula were powdered and then assayed using the High-Performance Liquid Chromatography (HPLC) method specified by the US Pharmacopeia for the assay of vitamin C [12].

Vitamin C and vitamin C + spirulina tablets were prepared at concentrations, 250 µg/mL and 50 µg/mL respectively. The solutions were then subjected to HPLC analysis. The diluent used was a solution of 0.56 g of EDTA in $H_2PO_4^-$, K^+(2.04 g/L).

The solvent was milliQ water. Using the equation of the calibration graph obtained from the Chemical Reference Substance (CRS) of vitamin C, the real content of vitamin C in the formulated tablets were determined.

Dissolution test

Samples were taken at different times, and then subjected to HPLC analysis. Table 2 below summarizes the operating conditions for the dissolution test.

Table 2: Summary of conditions for performing the dissolution test.

Test settings	Features
Dissolution medium	HCl (0.1 N)
Medium temperature	37 °C ± 2 °C
Sampling times	0, 20, 40, 60, 90, 120, 150, 180, 240 min
Test duration	4 h
Balls number	06
Medium volume per ball	900 mL
Number of samples per ball	01
Palette rotation speed	50 rpm
Dosage method	HPLC/DAD – USP Vitamin C assay
Dissolution medium sample volume	10 mL

High Performance Liquid Chromatography

The analysis was carried out on a Hitachi brand (VWR/Belgium) high-performance liquid chromatograph using a photodiode array detector. The operating conditions are summarized in the following Table 3 below.

Table 3: Summary of HPLC assay operating conditions.

Settings	Features
Column	C_{18}, (250 x 4 mm i.d; dp 5 µm particle size)
Mobile phase	Methanol: Phosphate buffer 2.04 g/L, pH 3 (5: 95, v/v)
Flow rate	0.7 mL/min
Injection volume	10 µL
Oven temperature	30 °C
Detection wavelength	254 nm

RESULT AND DISCUSSION

RESULTS

1. Formulation with matrix agents (HPMC, HEC and MC)

Following the compression, a rough and crumbly texture was observed with the tablets of formulas A2 and A3 but a smooth texture with formula A1. The tablets of Carbopol-based formulas (C1, C2, C3) were smooth in texture but sticky.

The tablets produced with Avicel and the matrix agents, namely HEC and MC (A2 and A3) had cracks and easily crumbled, unlike those obtained with Avicel and HPMC (A1) which had satisfactory macroscopic characteristics.

2. Disintegration test during formulation

The disintegration times for the three formulas, expressed in minutes, are shown in Table 4.

Table 4: Disintegration time in minutes for formulas A1, A2 and A3.

Formulas	A1	A2	A3
Disintegration time (min)	300	60	45

A1 (Avicel and HPMC); A2 (Avicel and HEC); A3 (Avicel and MC)

From Table 4, there is a clear superiority in the matrix agent HPMC compared to HEC and MC. The objective of our work was to prepare matrix tablets that would have sustained release properties evidenced by longer disintegration time relative to the simple tablets. It is evident from Table 4 that HPMC displayed the best matrix effect and was therefore chosen for the final formulation. We found that for the formulas containing Carbopol, there was swelling during the disintegration. Considering these results, the formula with Avicel and HPMC was the one chosen for the addition of spirulina.

3. Formulation with spirulina

Following the various tests to optimize the proportion between vitamin C and spirulina, formula S is presented in Table 5:

Table 5: Formula for incorporating Spirulina

	Formula S
	Proportions (%)
Vitamin C (20%)	32.7
Spirulina (80%)	
Starch	5.8
Avicel	14.5
Lactose	17.3
Mg Stearate	1.1
Talc	1.1
HPMC	27.2

Tablets only based on vitamin C as well as those based on spirulina and vitamin C are represented in figure 1:

Figure 1: (a): Vitamin C matrix tablets showing crumbling (Formula A1); (b): Spirulina and vitamin C matrix tablets (Formula S).

4. Friability test

Formula A1 tablets broke during testing which reflects their systematic non-compliance with the friability test. However, formula S tablets, did not show any breakage during the entire test. The total mass of the tablets A1 before and after tumbling were 6.93 g and 6.59 g respectively, corresponding to a mass loss of 4.9%. Therefore, the friability test of A1 did not comply with the European Pharmacopeia specifications because the mass loss is higher than 1%. These results demonstrate the higher strength of the spirulina and vitamin C tablets compared to those containing only vitamin C.

5. Mass uniformity

The results of the mass uniformity test are summarized in Table 6 below.

Table 6: Summary of the results of the mass uniformity test.

Tablet	Vitamin C tablets	Spirulin + vitamin C tablets
	Masses (mg)	Masses (mg)
1	410	660
2	410	660
3	420	670
4	400	680
5	400	660
6	390	670
7	410	660
8	400	650
9	420	650
10	410	680
11	390	680
12	410	660
13	400	670
14	390	660
15	410	660
16	410	670
17	400	660
18	410	660
19	410	680
20	400	660
Average	405	665
Maximum	420	680
Minimum	390	650
Standard deviation	8.8	9.45
CV	2.1	1.38

The average masses of vitamin C tablets (405 mg) and spirulina + vitamin C tablets (665 mg) are all greater than 250 mg. Table 7 presents the specifications of the mass uniformity test according to the European Pharmacopoeia and the compliance conclusions of the test.

Table 7: Mass uniformity test interpretation.

Tablets	Tablets average masse (mg)	5% deviation limit around the average mass	Conclusion (Compliant/Non-compliant)
Vitamin C	405 ± 8.8	[384.75 - 425.25]	Compliant
Spirulina + Vitamin C	665 ± 9.4	[631.75 - 698.25]	Compliant

6. Content uniformity test

Figures 2, 3, and 4 below show the chromatograms of blank and ascorbic acid (retention time 3.6 min) in the SCR and in a sample, respectively.

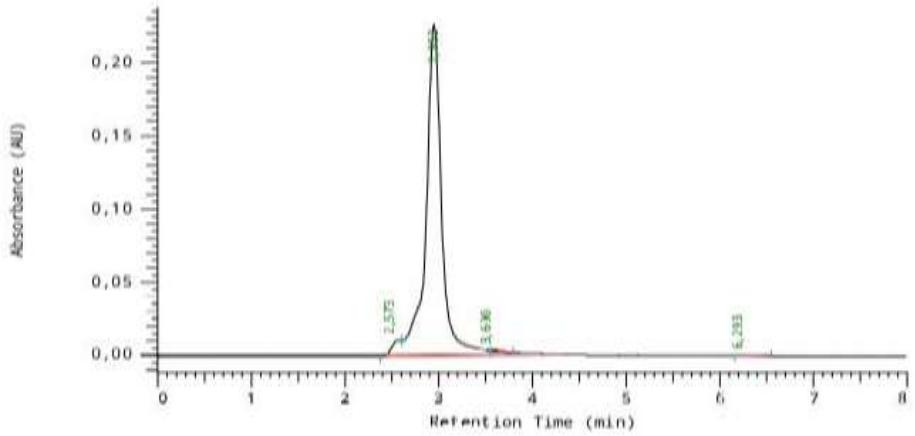

Figure 2: Blank chromatogram.

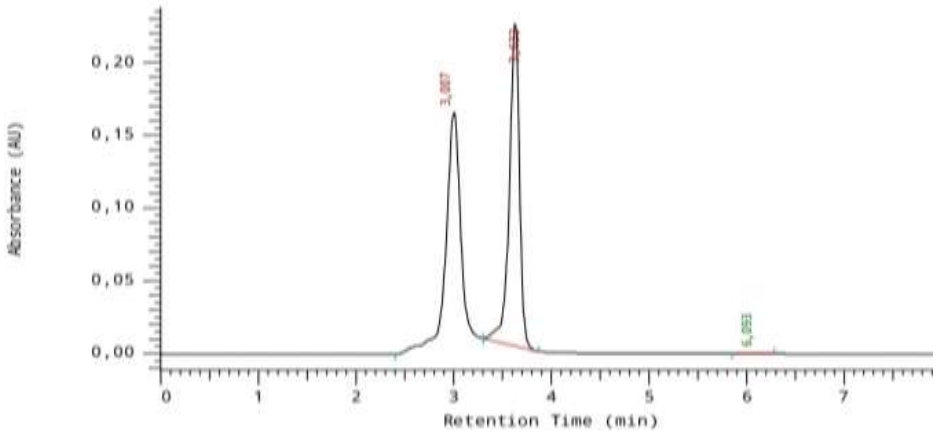

Figure 3: Chromatogram of ascorbic acid at 245 nm in SCR.

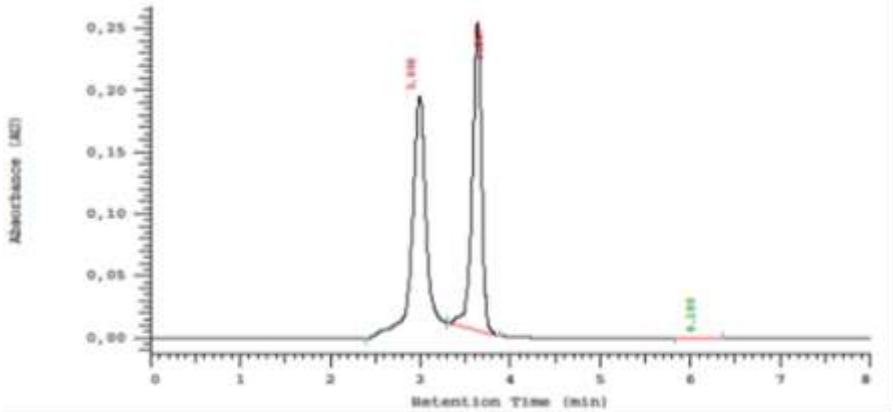

Figure 4: Chromatogram of ascorbic acid at 245 nm in a sample.

The content uniformity results are recorded in Table 8 below.

Table 8: Summary of the results of the content uniformity test.

Tablet	Vitamin C tablets		Spirulina + vitamin C tablets	
	Mass (mg)	Content (%)	Mass (mg)	Content (%)
1	200	114.1	37.1	89.9
2	201.2	114.8	42.8	101.9
3	167.7	95.7	42	101.9
4	159.4	91	36.1	87.5
5	161.1	91.9	41.3	100
6	170.3	97.2	42.4	102.7
7	170	97	37.1	89.9
8	174.7	99.7	46.1	111.7
9	159.9	91.2	44.2	107.1
10	187.3	106.9	44.2	107.1
Maximum	201.2	114.8	46.1	111.7
Minimum	159.4	91	36.1	87.5
Average	175.1	99.9	41.2	99.9
Standard deviation	15.7	8.9	3.4	8.2
CV	8.9	8.9	7.9	8.2

Table 9 presents the specifications of the content uniformity test according to the European Pharmacopoeia and the compliance conclusions of the test.

Table 9 Content uniformity test interpretation specifications.

Tablets	Content average	15% deviation limit around the average content	Conclusion (Compliant/Non-compliant)
Vitamin C	99.9 ± 8.9	[91.1 - 114.9]	Compliant
Spirulina + Vitamin C	99.9 ± 8.2	[87.7 -111.8]	Compliant

7. Dissolution test

Figure 5 compares the average dissolution profiles of vitamin C tablets and spirulina tablets combined with vitamin C.

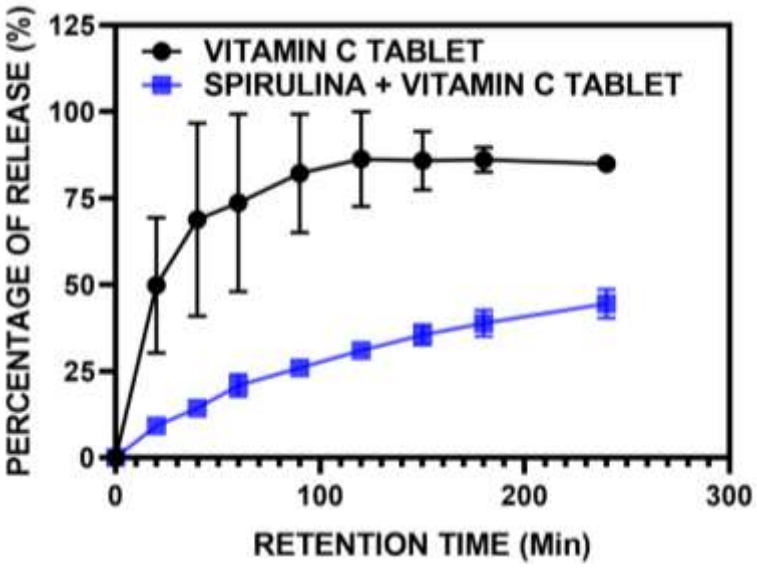

Figure 5: Comparison of average dissolution profiles of vitamin C tablets and spirulina + vitamin C tablets.

The dissolution profiles obtained show an increase in the release of vitamin C by the two types of tablets, which perfectly illustrates the sustained release property conferred by the matrix agent HPMC. The speed and rate of release of vitamin C from tablets A1 are higher than that of spirulina + vitamin C of tablets S, it could be explained by the higher concentration of vitamin C in Tablets A1 relative to Tablets S.

DISCUSSION

The macroscopic evaluation of the tablets revealed some cracks in certain vitamin C tablets, which is an indication of insufficient firmness. This was confirmed by the friability test which proved to be unsatisfactory. These observations can be explained by a low compression force or by the no granulation of the powders before compression. This result is similar to that of Catalin D. *et al*., who experimented the influence of powder particles size and shape on tablets formulation characteristics, particularly their friability [14]. The slight resistance of vitamin C tablets associated with spirulina could be explained by a better cohesion of the particles in the powder containing spirulina. Catalin D. *et al*., also demonstrated the influence of compression force, particle size and shape on tablets strength [15]. These parameters can be corrected by operations prior to compression such as the granulation and spheronization [14]. Furthermore, Saurav A. *et al*., proved that wet granulation applied to HPMC powder gives much more compact tablets [15].

The satisfactory results obtained for the mass uniformity test reflect good distribution of the mixture of powders in the tablets. However, it is noted that the masses and the contents of vitamin C of the vitamin C only tablets have a greater variation than the masses and the contents of the tablets of spirulina + vitamin C. This dispersion difference could evoke a better flow of the mixture of spirulina powder and vitamin C compared to the powder containing only vitamin C. This is because of the better particle size of the spirulina powder which through their addition to the vitamin C powder gave a better flow to the mixture. Yohei M. *et al*., demonstrated the negative influence of powders flow on tablets' masses variation [13]. Indeed, a poor flow of the powder bed as well as a significant cohesion of the powder hampers compressibility, which leads to significant variations in the tablets mass and consequently the active ingredient content [16].

The obtained tablets showed sustained release kinetics after the dissolution test. Indeed, after 4 hours under the same conditions, the quantity of vitamin C dissolved (in the case of vitamin C alone tablets) was practically double the quantity of vitamin C in the vitamin C + spirulina tablets. This high speed of release and the explosion (burst) observed in vitamin C tablets could be explained by the high content of vitamin C in these tablets, unlike tablets containing both spirulina and vitamin C. Indeed, as demonstrated by Yadav K. *et al*., a high concentration of the active ingredient increases its contact surface with the dissolution medium, causing a high rate of release and dissolution [17]. Since the dissolution rate is also a function of the disintegration time of the tablets, we noticed a more coordinated and time-dependent (sustained) release of vitamin C from the spirulina + vitamin C tablets. These results are similar to those of Saurav A. *et al*., who found that good sized particles give more compact matrices, which slows down the dissolution rate of the active ingredient [15]. A prior granulation operation of the powder of formula A1 can improve its flow and its compressibility and will make it possible to obtain a firmer matrix which will slow down the rate of release of vitamin C.

CONCLUSION

This preliminary work, for which the general objective was to formulate matrix tablets based on spirulina and vitamin C, has allowed the development of a particular formula of spirulina and vitamin C tablets with prolonged release of vitamin C and better absorption. The tablets were manufactured and the various pharmacopoeial tests were carried out for quality control. These are mass uniformity, friability, disintegration, content uniformity and dissolution tests. The dissolution test results revealed that 45% of vitamin C was dissolved after 4 hours, perfectly illustrating the sustained release characteristics conferred by the matrix agent. It should be noted, however, the need to improve the physical properties of powder mixtures before compression. The mixture of vitamin C and spirulina is therefore compressible and compatible with HPMC matrix agent.

ACKNOWLEDGMENTS

We would like to thank Dr Raphael Alolga for his time to review the English of this paper. We are grateful to Parfait Doffon and Eldad Wotto for the technical support.

CONFLICTS OF INTEREST

We declare there is no conflict of interest for the publication of this paper.

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