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Dissolution and spectrophotometric determination of astaxanthin in aqueous solutions

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The poor solubility of astaxanthin in water can cause problems during dissolution tests of dosage forms because they are usually performed in water-based media. The aim of this study was the development of a convenient dissolution medium and a method for a spectrophotometric determination of astaxanthin in an aqueous solution. Three surfactants in different concentrations were tested as solubility-improving substances: sodium lauryl sulfate (SLS), polysorbate 80 (PS 80) and macrogolglycerol hydroxystearate (Cremophor®RH 40, CR 40). Optimal conditions were determined. The dissolution of astaxanthin from solid dosage form is performed into 1000 g of a solution of sodium lauryl sulfate with the concentration 1.0% (w/w) at 37 °C by paddle method, 100 rotations per minute, dissolution time 30 minutes. The procedure is convenient for solid dosage forms with a content of 4 to 12 mg of astaxanthin. The spectrophotometric determination of astaxanthin in aqueous solution from the dissolution test is measured at 486 nm. The specific absorbance $A^{1\%}_{1cm}$ for astaxanthin in water is 2000, a sodium lauryl sulfate solution (1%) was used as a blank.

1. Introduction

Astaxanthin is a carotenoid belonging to a group of xanthophylles. They are produced in nature by plankton, algae and plants as well as a small number of bacteria and fungi. In plants and algae, carotenoids are actually part of the process of photosynthesis along with chlorophyll. Astaxanthin together with other carotenoids (e.g. beta-carotene, luteolin, zeaxathin) has an ability to interact with and neutralize chemically reactive oxygen species known as singlet oxygen and free radicals and it is called the world's strongest natural antioxidant. (Capelli et al. 2007)

In natural sources, astaxanthin is usually present in the form of esters with organic acids. The carotenoid fraction of *Haematococcus* algae (one of the main sources of astaxanthin) contains about 70% monoesters of astaxanthin, 10% diesters of astaxanthin, 5% free astaxanthin, with the remainder consisting of β-carotene, canthaxanthin, and lutein. Astaxanthin is usually determined by spectrophotometric or HPLC methods. Accurate quantification of esterified astaxanthin by HPLC is difficult, however, various systems have been developed and validated for the analysis of free astaxanthin. Thus, esterified astaxanthin must first be hydrolyzed by either a chemical or enzymatic procedure to yield all free astaxanthin (Lorenz 2001; Fuji Ver. 03). The spectrophotometric determination is not as accurate as HPLC, because it is also influenced by other carotenoids present in the samples. On the other hand it has some advantages over HPLC which often needs more complicated sample preparation, expensive instrumentation, and long analysis time. The spectrophotometric method is convenient and suitable for a rapid determination of many samples in a short time. The problem of spectra overlapping resulting from chemical analogues that cause false results could be solved to some extent by using a derivative ratio spectrophotometric method (Hui et al. 2005). Due to its poor solubility in water, astaxanthin is usually determined in an organic solvent environment. These methods are convenient e.g. for evaluation of samples during production of astaxanthin, evaluation of samples of feed for fish, etc. But the use of organic solvents is not suitable for measurement of dissolution of astaxanthin from final dosage forms (tablets, capsules), which appeared on the market to a considerable extent during last years.

The dissolution of active substance from the final dosage form is one of the most important quality tests. It is usually performed in water or various buffered solutions. Problem arises with an active substance hardly soluble or insoluble in water (e.g. astaxanthin). In such a case a suitable dissolution medium must be developed. If the solubility of the active substance depends on pH, a suitable buffer solution with a convenient pH value can be used. For substances with pH-independent solubility, the most often used method is an addition of surfactant to the dissolution media (Rong 2008; Noory et al. 2000). Surfactants that have been used in the FDA OGD's dissolution database include sodium lauryl sulfate, polysorbates, polyoxyethylene 10 laurylether, and others (FDA). The improvement of solubility of astaxanthin in water can be achieved also by complexation with cyclodextrines (Soyoung et al. 2008; Lockwood 2003).

2. Investigations, results and discussion

The dissolution of astaxanthin was measured in water solutions of sodium lauryl sulfate (SLS), polysorbate 80 (PS 80) and macrogolglycerol hydroxystearate (Cremophor RH 40, CR

Table 1: Absorbance of astaxanthin in a solution of sodium lauryl sulfate (SLS)

Concentration of SLS (%)	Average absorbance	Statistical significance*
0.0	0.388 ± 0.015	–
0.05	0.751 ± 0.012	YES
0.1	0.772 ± 0.012	YES
0.5	0.788 ± 0.011	YES
1.0	0.796 ± 0.031	NO
3.0	0.806 ± 0.025	NO
5.0	0.808 ± 0.032	NO

* Statistical significance of the difference between two subsequent measurements

40) with different concentrations. Spectrophotometric determination of astaxanthin is usually performed in organic solvents (n-hexane, petroleum ether, acetone) at wavelenght 470–480 nm and with a specific absorbance $A^{1\%}_{1cm} = 2100$ (Lorenz 2001; Fuji Ver. 03; Barbosa et al. 1999; Johnson et al. 1994; Tolasa et al. 2005). There is no data regarding the absorption spectrum, absorption maximum and specific absorbance for spectrophotometric determination of astaxanthin in aquoeus solutions of surfactants in the literature. That is why the principles of this experiment were: (1) measurement of absorption spectrum and absorption maximum of solutions of astaxanthin in 1% (w/w) solution of each surfactant, (2) measurement of absorbance of solutions of astaxanthin in water with increasing concentration of surfactant (the concentration of surfactant in a solution, where the absorbance stopped changing, was considered as sufficient for dissolution of all amount of astaxanthin), (3) plotting of a calibration curve, calculation of the equation of a regression straight line and calculation of a specific absorbance.

The maximal absorbance of astaxanthin in solutions of SLS, PS 80 and CR 40 (4 mg of astaxanthin in 1000 g of 1% solution of surfactant) was at 486 nm, 491 nm and 492 nm, respectively. Tables 1, 2 and 3 quote the absorbance of astaxanthin in solutions of surfactants with different concentration.

In the case of SLS the absorbance increased from the beginning, but the differences in solutions with the concentration of surfactant 1% and higher were not statistically significant. The dependence between absorbance and concentration of surfactant in the case of PS 80 was not unambiguous. The absorbance of solutions increased from the beginning, but at higher concentrations of PS 80 it again decreased. It is possible, that astaxanthin reacts with PS 80 or with some impurity in it resulting in the decrease of astaxanthin content in the solution. Besides the absorbance of astaxanthin in PS 80 solutions was approximately only half of absorbance in the solution of SLS.

In the case of CR 40 the absorbance of astaxanthin solutions increased all the time together with the increase of surfactant

Table 2: Absorbance of astaxanthin in a solution of polysorbate 80 (PS 80)

Concentration of PS 80 (%)	Average absorbance	Statistical significance*
0.0	0.367 ± 0.005	–
0.05	0.435 ± 0.010	YES
0.1	0.458 ± 0.015	YES
0.5	0.461 ± 0.006	NO
1.0	0.447 ± 0.014	YES
3.0	0.423 ± 0.008	YES
5.0	0.364 ± 0.011	YES

* Statistical significance of the difference between two subsequent measurements

Table 3: The absorbance of astaxanthin in a solution of macrogolglycerol hydroxystearate (Cremophor RH 40, CR 40)

Concentration of CR 40 (%)	Average absorbance	Statistical significance*
0.0	0.367 ± 0.012	–
0.05	0.434 ± 0.017	YES
0.1	0.458 ± 0.014	YES
0.5	0.652 ± 0.023	YES
1.0	0.679 ± 0.010	YES
3.0	0.686 ± 0.020	NO
5.0	0.684 ± 0.027	NO

* Statistical significance of the difference between two subsequent measurements

concentration and the differences were statistically significant until the concentration of surfactant reached 3.0%. The absorbance was lower when compared with corresponding solutions of SLS and it stabilized at higher concentration of CR 40, that is why sodium lauryl sulfate was chosen as the most suitable surfactant for the dissolution of astaxanthin.

Values of concentration of astaxanthin and corresponding absorbances necessary for plotting of a calibration curve and calculation of a specific absorbance are listed in Table 4.

The optimal conditions for dissolution and spectrophotometric determination of astaxanthin (method 3.2.4) were used for testing the method for 8 and 12 mg of astaxanthin respectively. No statistically significant differences between theoretical and measured concentration of astaxanthin in solutions were found (Table 5).

The optimal conditions for dissolution of astaxanthin to water-based medium from solid dosage forms with the content of active substance from 4 to 12 mg were determined. The dissolution was performed into 1000 g of a solution of sodium lauryl sulfate with the concentration 1,0% (w/w) at 37 °C by the paddle method, 100 rotations per minute, dissolution time 30 min. After this period of time all the amount of active substance is dissolved. Simultaneously the method of a spectrophotometric determination of astaxanthin in water solutions was developed. The prepared solution from dissolution test is filtered and it's absorbance is measured at 486 nm. The concentration of active substance is calculated with the help of a specific absorbance $A^{1\%}_{1cm} = 2000$ for a water-based solution of astaxanthin. Solution of sodium lauryl sulfate 1% (w/w) is used as a blank.

3. Experimental

3.1. Material

Astaxanthin (BioAstin®, Tablet Grade Beadlets) was purchased from Cyanotech, U.S.A. via Faravelli s.r.o., Czech republic. The product is a microencapsulated lipid extract of Haematococcus pluvialis microalgae with known content of astaxanthin (HPLC). In experiments, the amount corresponding to 4 mg of pure astaxanthin was used (majority of products on the market contain 4 mg of astaxanthin in an individual dose). Sodium lauryl sulfate purchased from Centralchem s.r.o., Slovakia in p.a. grade was used. Polysorbate 80 complying with Ph.Eur. requirements was purchased from Interpharm a.s., Slovakia. Macrogolglycerol hydroxystearate (Cremophor RH 40) was purchased from BASF Slovensko, spol. s r.o., Slovakia. Dissolution tester Erweka DT 6, Germany was used for a dissolution of astaxanthin. Spectrophotometer Unicam Helios Gamma, England was used for a measurement of absorbance.

3.2. Methods

3.2.1. Measurement of absorption spectrum and absorption maximum

1000 g of 1% (w/w) solution of each surfactant (SLS, PS 80, CR 40) in purified water was prepared. The amount of BioAstin corresponding to 4 mg of pure astaxanthin was disolved in the solution of surfactant. The dissolution

Table 4: Values for plotting of a calibration curve and calculation of a specific absorbance

Concentration of astaxanthin (%)	Absorbance	Equation of a regression straight line	Regression coefficient R^2	Specific absorbance $A^{1\%}{}_{1cm}$
$(5.00 \pm 0.006) \times 10^{-5}$	0.098 ± 0.002	$y = 0.0005x$	$R^2 = 0.9999$	$E^{1\%}{}_{1cm} = 2000$
$(1.00 \pm 0.001) \times 10^{-4}$	0.198 ± 0.001			
$(1.50 \pm 0.002) \times 10^{-4}$	0.296 ± 0.002	where		
$(2.00 \pm 0.003) \times 10^{-4}$	0.392 ± 0.003			
$(2.50 \pm 0.003) \times 10^{-4}$	0.496 ± 0.003	y = concentr. of		
$(3.00 \pm 0.004) \times 10^{-4}$	0.588 ± 0.005	astaxanthin (%)		
$(3.50 \pm 0.005) \times 10^{-4}$	0.689 ± 0.011			
$(4.00 \pm 0.005) \times 10^{-4}$	0.788 ± 0.004	x = absorbance in		
$(4.50 \pm 0.006) \times 10^{-4}$	0.890 ± 0.008	1 cm cuvette		
$(5.00 \pm 0.007) \times 10^{-4}$	0.989 ± 0.004			

Table 5: Spectrophotometric determination of astaxanthin

Amount of astaxanthin (mg/1000 g of solution)	Absorbance	Theoretical concentration[3](%)	Measured concentration[4](%)	Statistical signification of the difference
4	0.796 ± 0.003	$(4.00 \pm 0.010) \times 10^{-4}$	$(3.98 \pm 0.020) \times 10^{-4}$	NO
8	0.794 ± 0.002[1]	$(4.00 \pm 0.035) \times 10^{-4}$	$(3.97 \pm 0.024) \times 10^{-4}$	NO
12	0.793 ± 0.002[2]	$(4.00 \pm 0.029) \times 10^{-4}$	$(3.96 \pm 0.031) \times 10^{-4}$	NO

[1] Sample diluted 1:1 with 1% solution SLS before measurement of absorbance.
[2] Sample diluted 1:2 with 1% solution SLS before measurement of absorbance.
[3] Calculated from weighed amount of astaxanthin and after dilution (where applicable).
[4] Calculated from an absorbance and an equation of a regression straight line.

was performed in a vessel of dissolution tester Erweka DT 6 at 37 °C, using a paddle with 100 rotation per minute, dissolution time 30 minutes. The solution was filtered through a filter paper and used for absorbance measurement. A solution of pure surfactant with the concentration 1% (w/w) was used as a blank.

3.2.2. Measurement of absorbance of an aqueous solution of astaxanthin containing surfactants

Solutions of surfactants in water with the concentrations 0.05, 0.1, 0.5, 1.0, 3.0 and 5.0% (w/w) were prepared. The amount of BioAstin corresponding to 4 mg of pure astaxanthin was disolved in 1000 g of each solution. The dissolution was performed in a vessel of dissolution tester Erweka DT 6 at 37 °C, using a paddle with 100 rotation per minute, dissolution time 30 minutes. Each sample was filtered through a filter paper and it's absorbance was measured at a wavelenght of maximum absorbance (the result of method 3.2.1). A solution of pure surfactant with the corresponding concentration was used as a blank. Six parallel measurements were performed with each concentration of surfactant. A statistical significance of a difference between absorbances of each subsequent measurements was calculated ($\alpha = 0{,}05$).

3.2.3. Calibration curve and calculation of specific absorbance

The conditons of a measurement, which were found as optimal during previous experiments, were used. Solutions of astaxanthin (BioAstin) with expected absorbance 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 were prepared by dissolving of the appropriate amount of active substance in a solution of SLS with the concentration 1% (w/w). The dissolution was performed in a vessel of dissolution tester Erweka DT 6 at 37 °C, using a paddle with 100 rotation per minute, dissolution time 30 minutes. The solutions were filtered through a filter paper and their absorbance was measured at 486 nm. A 1% (w/w) solution of pure SLS was used as a blank. Three parallel measurements were performed with each concentration of astaxanthin. The calibration curve was plotted from obtained values and an equation of a regression straight line was calculated. The specific absorbance $A^{1\%}{}_{1cm}$ was calculated from the equation.

3.2.4. Testing the method for 8 and 12 mg astaxanthin

The developed method for a dissolution and spectrophotometric determination of astaxanthin was tested for 8.0 and 12.0 mg of astaxanthin. The amount of BioAstin corresponding to 4 mg (8 mg, 12 mg) of pure astaxanthin was disolved in 1000 g of a 1% (w/w) water solution of SLS. The dissolution was performed in a vessel of dissolution tester Erweka DT 6 at 37 °C, using a paddle with 100 rotation per minute, dissolution time 30 min. The solution was filtered through a filter paper, diluted with 1% solution of SLS in the ratio 1:1 and 1:2 for amounts of astaxanthin 8 and 12 mg respectively and it's absorbance was measured at 486 nm. A 1% (w/w) solution of pure SLS was used as a blank. Three parallel measurements were performed. The specific absorbance calculated in previous experiment (method 3.2.3) was used for a calculation of astaxanthin content and a theoretical and measured concentration of astaxanthine were compared.

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