IMMEDIATE-RELEASE SOLID DOSAGE FORM WITH β-CAROTENE. I. PREFORMULATION STUDIES

LĂCRĂMIOARA POPA, MIHAELA VIOLETA GHICA

Department of Physical-Chemistry, Faculty of Pharmacy, 6 Traian Vuia str., Bucharest, Romania

Abstract. The objective of this study was to perform preformulation assays (solubilization and stability) addressed to β-carotene, aiming at a further immediate-release solid oral dosage form. The carotenoidic pigment was solubilized in water by linear water-soluble macromolecules. There was formed a solid dispersion of β-carotene with different polymers. The kinetics of β-carotene oxidation in the aqueous solutions resulted were determined in the presence and in the absence of ascorbic acid (vitamin C) as antioxidant. The influence of polymer species was established. A factorial 3^2 experimental design completed with graphical analysis of response surface was developed in order to determine the protective action of ascorbic acid against β-carotene degradation and to optimize the solid dispersion formulation.

Keywords: β-carotene, water solubilization, stability, ascorbic acid, optimization.

INTRODUCTION

It is well known and widely demonstrated the antioxidant role of carotenoids (especially β-carotene) in the prevention of several cancers, cataracts, and in a non-invasive management of coronary heart diseases [2, 6, 7, 12]. In previous papers we demonstrated through QSAR methods (molecular topology analysis and molecular connectivity methods) the direct implication of this low toxicity compound in cardiovascular therapy [4, 5].

β-carotene presents several formulation problems: low bioavailability, water insolubility and a great instability due to its non-saturation degree. It is decomposed, in the presence of light and atmosphere oxygen, into inactive products (epoxy-compounds) with visible changes in pigment color [3]. Based on these reasons there were developed preformulation studies focused on β-carotene solubilization and stability. The further formulation studies would be developed in order to design, evaluate and optimize an immediate-release β-carotene solid oral dosage form.

Received December 2003;
in final form December 2004.

ROMANIAN J. BIOPHYS., Vol. 13, Nos. 1–4, P. 65–74, BUCHAREST, 2003

In our study it was applied a technique for β-carotene water solubilization through linear macromolecules [8]. The solid dispersion of β-carotene with different polymers was dissolved in water and the kinetics of β-carotene oxidation in these aqueous solutions was determined (generally, reactions in solid phase are too slow and aqueous phase reaction is preferred for drug stability studies). β-carotene will be associated in the solid dosage form with ascorbic acid, a powerful reducing agent, antioxidant and protective substance. Ascorbic acid has also a synergetic potentate action with β-carotene in preventing oxidation of low-density lipoproteins, with great implications in cardiovascular therapy and it is considered one of the most active antioxidants in extra-cellular fluids [5].

With the development of a factorial experimental design (3^2) completed with a response surface analysis, the optimum content of ascorbic acid for an effective protective action against β-carotene oxidation was evaluated .

MATERIALS AND METHODS

MATERIALS

β-carotene, Poly (N-vinylpyrrolidone) PVP K_{90}: molecular weight –mol.wt.– 360,000; Polyethylene glycol PEG_{6000}: average mol.wt. 5,000–7,000; Poly (vinyl alcohol) PVA: mol.wt.m 75,680; Ascorbic acid was purchased Merck, Germany. Solvents: chloroform, acetone, ethanol were of analytical grade.

METHOD FOR PREPARATION OF A β-CAROTENE WATER SOLUBLE PRODUCT

There was prepared a solid dispersion of β-carotene with polymers PEG, PVP and PVA by using the co-solvent method. The composition of the solid dispersions and of the solvents used is shown in Table 1.

Table 1

Composition of β-carotene/polymer solid dispersions and solvents used at solubilization

Solid dispersion	β-Car* (mg)	Solvent (ml)	Polymer (g)	Solvent (ml)	Mole ratio polymer/βCar*
β-Car*/ PEG	0.50	1.50 ($CHCl_3$)	1.18	3.00($CHCl_3$)	64.00
β-Car*/ PVP	0.50	1.50 ($CHCl_3$)	0.19	4.00($CHCl_3$)	5.00
β-Car*/ PVA	0.50	10.00(acetone)	1.50	10.00 (water)	21.00

β-*Car = β-carotene

An organic solvent containing β-carotene was added to an organic solution of polymer. The mixed solutions were evaporated to a dry β-carotene/polymer film under a reduced pressure at room temperature. By adding a small amount of water

and gently stirring at room temperature, the film was transformed in a homogeneous paste that was then diluted with water to a desired concentration of β-carotene. These aqueous solutions of β-carotene with polymers were used in the next assays.

Observation: It was selected the same organic solvent (chloroform) for polymers PVP and PEG, as well as for β-carotene, in the minimum quantities required for its total and rapid elimination. The solvent acetone was used as organic solvent for β-carotene in the solid dispersion with PVA for its miscibility with water (the solvent used for polymer).

There were also prepared solid dispersions of polymer/β-carotene/ascorbic acid by adding an amount of ascorbic acid in absolute alcohol to the dried β-carotene/polymer films before water solubilization. The ratio of ascorbic acid/ β-carotene (1:1) corresponding to a concentration of ascorbic acid acting as antioxidant was selected after preliminary assay [1].

ANALYTICAL METHOD AND KINETIC ANALYSIS

The aqueous solutions of β-carotene with different polymers, with/without ascorbic acid, as described above, have served to the quantitative spectrophotometer assay in formulation stability study. Absorption spectra at room temperature were measured with a double beam spectrophotometer Lambda 2 (Perkin Elmer, Cole Parmer). Absorption maxima of β-carotene were determined at 410 nm, 459 nm, and 466 nm for PEG/carotene, PVP/carotene, and PVA/carotene, respectively [8]. At these absorption maxima for β-carotene, the ascorbic acid does not present absorption maxima or non-specific absorption at the levels of concentrations from the analyzed solutions. In these conditions the apparent rate constant of the reaction of β-carotene oxidation in aqueous solutions is given according to the terms of pseudo-first order kinetics by the following relation:

$$k_a = \frac{1}{t_i} \ln \left(\frac{A_0 - A_\infty}{A_i - A_\infty} \right) \tag{1}$$

where C_0 is the initial β-carotene concentration in aqueous solutions, and C is the concentration remaining at time t_i; $C_0 \sim A_0 - A_\infty$, and $C \sim A_i - A_\infty$, where A_0, A_i and A_∞ represent β-carotene absorbance at λ_{max}, at initial time t_0, after a time t_i, and at the time of complete drug photolysis, respectively.

There were carried out kinetic experiments of β-carotene oxidation in aqueous solutions obtained from the solid dispersion with PEG 6000, PVP K_{90}, and PVA polymers; with/without ascorbic acid. These stability experiments were developed in the absence of white light radiation (samples kept in dark conditions).

There were also computed other biopharmaceutical parameters with preformulation significance half-life: $t_{1/2} = \frac{0.693}{k_a}$ and shelf-life: $t_{90} = \frac{0.105}{k_a}$.

RESULTS AND DISCUSSION

EFFECT OF POLYMER TYPE ON THE β-CAROTENE OXIDATION RATE

In Figure 1 are presented the kinetic profiles for β-carotene oxidation in aqueous solutions prepared from solid dispersion, with/without ascorbic acid as antioxidant agent. The value of β-carotene apparent oxidation rate decreased in the order PVA > PEG > PVP.

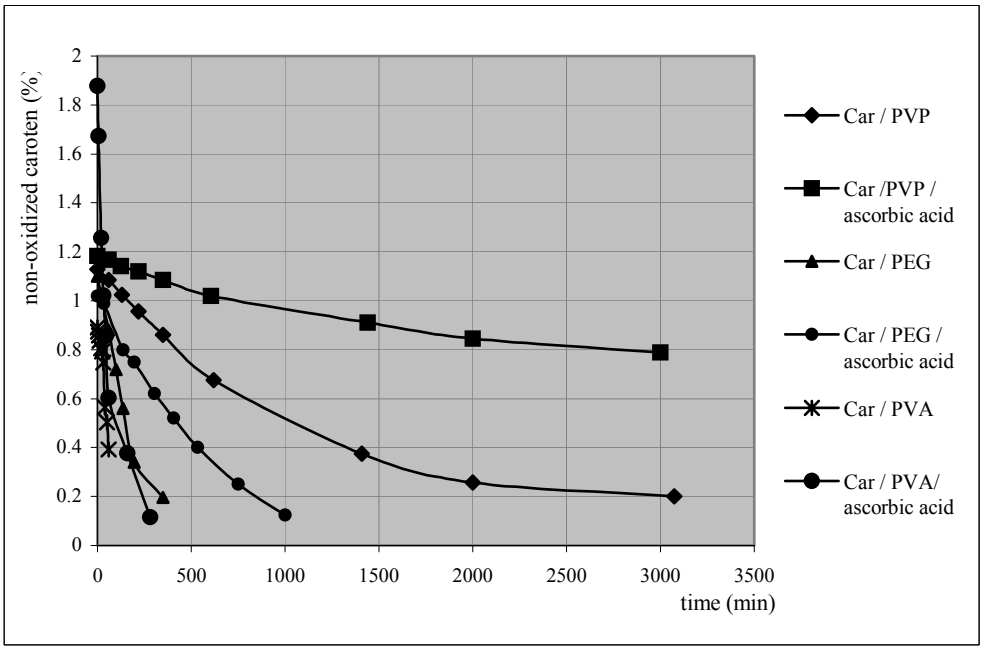

Fig. 1. – Oxidation of β-carotene in aqueous solutions from different solid dispersions.

In Table 2 are presented the values of the biopharmaceutical parameters with significance in β-carotene pharmaceutical preformulation tests: the apparent constant rates of β-carotene oxidation (k_a), the half-life ($t_{1/2}$), the shelf-life (t_{90}), and the percentage of non-oxidized β-carotene after 60 min (C_{60}). β-carotene in aqueous PVA solution was decomposed almost completely after 45 min, while β-carotene in aqueous PVP solution remained 98.4% non-oxidized after 60 min.

Table 2

Synthesis of β-carotene stability kinetic experiments in aqueous solutions, prepared from different polymers

Aqueous solutions*	k_a (10^{-4} min^{-1})	$t_{1/2}$ (min)	t_{90} (min)	C_{60} (%)
β-carotene/PVP	5.00	1386.00	210.00	1.60
β-carotene/PVP/ascorbic acid	1.80	3850.00	583.33	0.90
β-carotene/PEG	13.00	533.20	80.77	11.30
β-carotene/PEG/ascorbic acid	10.00	693.15	10.50	9.10
β-carotene/PVA	149.00	46.52	70.47	100.00
β-carotene/PVA/ascorbic acid	85.00	81.55	12.35	45.81

*without white light irradiation (samples kept in dark conditions).

The addition of ascorbic acid as antioxidant (reducing agent for β-carotene in aqueous solutions) leads to a near 3-time decrease in rate of drug decomposition (more evident for PVP solution) in the absence of white light radiation. Ascorbic acid has an active role in β-carotene protection against oxidation induced by aqueous oxygen. For these reasons we reached an optimum content of ascorbic acid in solid dispersion.

EXPERIMENTAL DESIGN

It was selected the most stable dispersion: β-carotene/PVP/ascorbic acid, with the above mentioned conditions of preparation and the corresponding aqueous solution. There was constructed a 3^2 randomized full factorial design for the kinetic experiments of β-carotene oxidation. An amount of hydrogen peroxide was added to the aqueous solution, in order to release supplementary oxygen and to develop oxidation experiments in critical conditions. The independent variables selected were X_1 = the concentration of ascorbic acid (mg/100 mg β-carotene), and the X_2 = the oxygen excess, as supplementary added hydrogen peroxide (% v/v). The effect of mentioned variables was investigated on the following system responses: oxidation rate of β-carotene k_a (Y_1), shelf-life t_{90} (Y_2) and the percentage of non-oxidized β-carotene after 60 min C_{60} (Y_3). The levels of independent variables, the design layout are shown in Table 3. There were also carried out six other experiments (outer array) corresponding to the real zero level for each independent variable [10]. That allowed us to study noise factors and to evaluate the range of the system responses in the absence of the antioxidant and without supplementary oxygen addition. The system responses were measured and computed, as presented in Table 3.

Table 3

Full factorial experimental design layout

Exp. no.	Variable level (in coded levels)		Dependent variable		
	X_1	X_2	Y_1	Y_2	Y_3
1	0.100	1.000	1.270	82.677	91.363
2	0.100	3.000	3.180	33.019	82.508
3	0.100	5.000	6.000	17.213	73.927
4	0.200	1.000	0.380	276.316	98.165
5	0.200	3.000	0.640	164.063	96.273
6	0.200	5.000	0.700	150.000	95.683
7	0.300	1.000	0.150	700.000	99.096
8	0.300	3.000	0.250	420.000	98.494
9	0.300	5.000	0.330	318.182	98.189
10	0.000	1.000	6.600	15.909	65.494
11	0.000	3.000	12.600	8.333	47.665
12	0.000	5.000	18.300	5.738	36.006
13	0.100	0.000	0.200	525.000	98.049
14	0.200	0.000	0.286	367.133	98.570
15	0.300	0.000	0.099	1065.990	99.090

Translation of coded levels in actual units

Coded level	1	0	1
X_1: g ascorbic acid	0.100	0.200	0.300
X_2 : % hydrogen peroxide	1	3	5

STATISTICAL APPROACH

A statistical model incorporating interactive and polynomial terms was utilized to evaluate the response and the relationships between variables:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2 \quad (2)$$

where Y is a dependent variable (k_a, t_{90}, C_{60}), b_0 is the arithmetic average of 15 responses and b_i is estimated coefficient for the factor X_i. The X_1X_2 and X_i^2 are the interaction and polynomial terms respectively. Carrying out multiple linear regressions in order to explain the effect of independent variables on selected responses generated polynomial equations (full model) eq. (2).

In order to identify the dominant factors affecting the variables k_a, t_{90} and C_{60} the data were analyzed by applying Student t-test. The terms showing the least absolute t-value were omitted from the regression equation and the remained terms are statistically significant. There were obtained polynomial equations for each dependent variable (reduced model) as follows:

$$k_a(Y_1) = 4.686 - 56.136X_1 + 1.757X_2 + 6.488X_1X_2 + 143.141X_1^2 \quad (3)$$

$$t_{90}(Y_2) = 330.220 - 956.576X_1 - 204.524X_2 + 8966.44X_1^2 + 26.282X_2^2 \quad (4)$$

$$C_{60}(Y_3) = 72.45 + 303.74X_1 - 7.972X_2 + 25.9X_1X_2 - 737.86X_1^2 + 0.17X_2^2 \quad (5)$$

The multiple regressions analysis and ANOVA were performed with Statistical Analysis System (SAS), and the results are summarized in Table 4.

Table 4

Summary of ANOVA analysis and t-Student test for the measured responses

Response Y_i	Degree of freedom (df)	F-Snedecor	p	Standard error (SE)	Student test value t (10)
k_a	(4, 10)	21.04	0.000074	1.648	6.37
t_{90}	(4, 10)	20.04	0.000900	117.210	3.57
C_{60}	(5, 9)	9.94	0.000100	6.347	3.82

OPTIMIZATION STUDY

The aim of these experiments was to evaluate the protective activity of ascorbic acid against β-carotene oxidation in aqueous solutions prepared with solid dispersion of β-carotene/PVP/ascorbic acid.

There were find preformulation parameters in order to minimize the rate of oxidation: variable k_a (Y_1) and to maximize both shelf-time t_{90} (Y_2) and the percentage of non-oxidized β-carotene after 60 min: C_{60} (Y_3).

In order to determine the influence of the independent variables X_1 and X_2 upon each of the dependent variables, there were developed mathematical and graphical approaches. The response surfaces and contour plots are represented in Figures 2 and 3.

For locating an optimum pair of values (X_1, X_2), a test for a local maximum or minimum of Y_i (X_1, X_2) in terms of partial derivatives was applied [9, 11]. The derivatives of Y_i with respect to X_1 and X_2 were computed and fit to zero. The function Y_1 may accept a geometrical locus of minimum, a ridge of minimum (Figure 2b), with the equation:

$$286.282X_1 - 6.488X_2 - 56.136 = 0 \quad (6)$$

that has the solution $X_1 = 0.200$, $X_2 = 0.000$, with significance for solid dispersion preformulation and future β-carotene formulation studies. Without supplementary addtion of oxygen, the optimum concentration of ascorbic acid in PVP/β-carotene solid dispersion is of 200 mg ascorbic acid/100 mg β-carotene. In this case, applying eq. (3), the theoretical value of constant rate is zero.

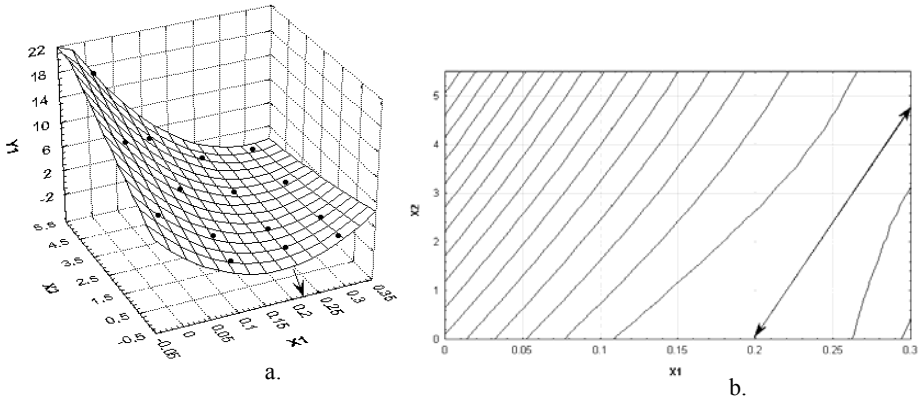

Fig. 2. – Response surface for the variable k_a (β-carotene oxidation rate): a. computed from eq. (3) and b. contour plot with the ridge of minimum, according to eq. (6).

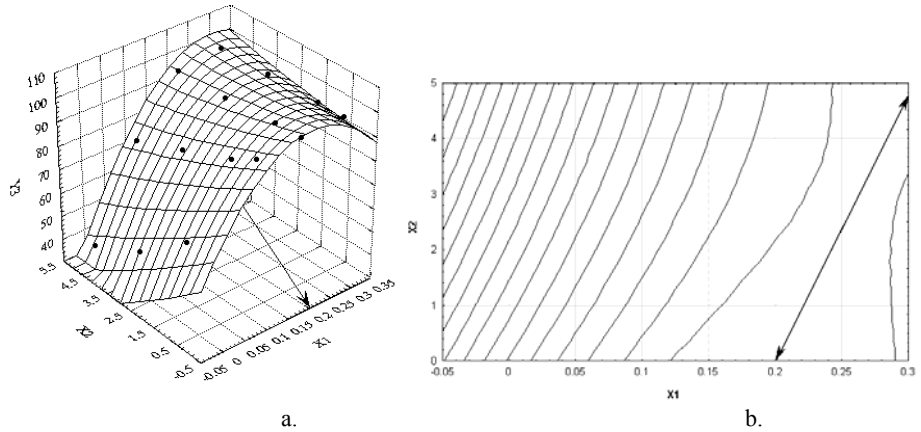

Fig. 3. – Response surface for the variable C_{60} (non-oxidized β-carotene after 60 min.): a. computed from eq. (5) and b. contour plot for the variable C_{60}, with the ridge of maximum, according to eq. (7).

The same procedure was applied to find the optimum ascorbic acid concentration that maximizes the percentage of non-oxidized β-carotene, by using eq. (5). The function C_{60} (Y_3) has a geometrical locus of maximum, a ridge with the following form:

$$303.742 + 25.898X_2 - 1475.72X_1 = 0 \quad (7)$$

as is shown in Figure 3b.

There was reached the same pair of values (0.200, 0.000) for the independent variables. It should be noted that in this case, the theoretical value for variables C_{60} was 100% – eq. (5).

Referring to eq. (4), the function t_{90} (Y_2) has neither a relative maximum nor a geometrical locus of maximum and it could not be included in an optimization study.

The ratio of 200 mg ascorbic acid/100 mg β-carotene, (2:1), in the PVP solid dispersion leads to a minimum drug oxidation rate. This value will be used in further pharmaceutical formulation design and studies for an immediate-release solid dosage form with β-carotene.

CONCLUSIONS

This study demonstrates that solid dispersions of β-carotene in several linear water-soluble macromolecules (like PVP), associated with ascorbic acid (vitamin C), can be a solution to solve some of β-carotene formulation problems (as solubility, stability). By combining an experimental design well known for optimization with a mathematical treatment for graphical analysis of the system responses, it was possible to identify the formulation conditions leading to an optimal and stable product.

The systematic preformulation approach enables us to further design and develop an immediate-release oral solid dosage form with β-carotene. The methodology described above can be applied to any preformulation and formulation study that involves different predefined characteristics.

Acknowledgements. The authors would like to thank Prof. Dr. St. Moisescu, for his helpful suggestions and comments on the optimization study, and to Merck Company, Bucharest for kindly supplying the substances.

REFERENCES

1. *******Handbook of pharmaceutical excipients*, Pharmaceutical Press, London, 1994.
2. AVRIEL, E., Y. APPELBAUM, G. URETZI, The effect of carotenoids on myocardial reperfusion-induced arrhythmias and injury, *J. Moll. Cell. Cardiol.*, 1988, **20**, 2–10.

3. CUSSON, C., A. SAVEROT-DAUVERGNE, T. GOUSSON, *Caroténoïdes. Le statut vitaminique: physiopathologie, exploration biologique et intérêt clinique*, Ed. Medicales internationales, Cachan, 1998.
4. GARCÍA-DOMENECH, R., CAROLINA GREGORIO-ALAPONT, J.V. JULIAN-ORTIZ, J. GÁLVEZ, LĂCRĂMIOARA POPA, Molecular connectivity to find β blockers with low toxicity, *Bioorg. & Med. Chem. Letters*, 1996, **5**, 567–572.
5. GARCÍA-DOMENECH, R., CAROLINA GREGORIO-ALAPONT, J.V. JULIAN-ORTIZ, J. GÁLVEZ, LĂCRĂMIOARA POPA, Pharmaceutical distribution diagrams: a tool for "de novo" drug design, *J. Molecular Graphics*, 1996, **14**, 272–276.
6. GERSTER, HERTA, Potential role of beta-carotene in the prevention of cardiovascular disease, *Int. J. Vit. Res*., 1991, **61**, 277–291.
7. GILLIGAN, D., M. SACK, V. GUETTA, P. CASINO, A. QUYYUMI, D.J. RADER, J.A. PANZA, R. CANNON, Effect of antioxidant vitamins on low density lipoproteins oxidation and hypercholesterolemia, *J. Am. Coll. Cardiol.*, 1994, **7**, 1611–1617.
8. ISAMU, I., I. MASAYUKI, Solubilisation of beta-carotene in water by water-soluble linear macromolecules, *Bull. Chem. Soc. Jpn.*, 1989, **62**, 1671–1673.
9. POPA, LĂCRĂMIOARA, *Metodologia cercetării ştiinţifice în domeniul farmaceutic*, Printech ed., Bucharest, 2003.
10. SCHWARTZ, J.B., R.E. O'CONNOR, Optimization techniques in pharmaceutical formulation and processing, in: *Modern pharmaceutics*, pp. 727–751, G.S. Banker and C.T. Rhodes, ed., Marcel Dekker, New York, 1996.
11. STEIN, S.K., *Calculus and analytic geometry*, McGraw-Hill Book Company, New York, 1973.
12. TOMA, J., E. ALBANESE, R. PALUMBO, G.C. NICOLO, P.E. MANGIANTE, S. BONATTI, R. CONCEDDA, L. SONTI, Effects in vitro of β-carotene on human oral keratinocytes from precancerous lesions and squamous carcinomas, *Anticancer. Drugs*, 1991, **6**, 581–589.