ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
30 (2002) 87–97

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

Experimental substantiation of the possibility of developing selenium- and iodine-containing pharmaceuticals based on blue–green algae *Spirulina platensis*

L.M. Mosulishvili [a], E.I. Kirkesali [a], A.I. Belokobylsky [a], A.I. Khizanishvili [a], M.V. Frontasyeva [b,*], S.S. Pavlov [b], S.F. Gundorina [b]

[a] *E.L. Andronikashvili Institute of Physics, Georgian Academy of Sciences, Tamarashvili str. 6, 380077 Tbilisi, Georgia*
[b] *Frank Laboratory of Neutron Physics, Joint Institute for Nuclear Research 141980 Dubna, Moscow Region, Russia*

Received 9 May 2001; received in revised form 5 March 2002; accepted 1 April 2002

Abstract

The great potential of using blue–green algae *Spirulina platensis* as a matrix for the production of selenium- and iodine-containing pharmaceuticals is shown experimentally. The background levels of 31 major, minor and trace elements (Na, Mg, Al, Cl, K, Ca, Sc, V, Cr, Mn, Fe, Co, Ni (using (*n*,*p*) reaction), As, Br, Zn, Rb, Mo, Ag, Sb, I, Ba, Sm, Tb, Tm, Hf, Ta, W, Au, Hg, Th) in *S. platensis* biomass were determined by means of epithermal neutron activation analysis. The dependence of selenium and iodine accumulation in spirulina biomass on a nutrient medium loading of the above elements was characterized. To demonstrate the possibilities of determining toxic element intake by spirulina biomass, mercury was selected. The technological parameters for production of iodinated treatment-and-prophylactic pills are developed.

Keywords: Blue–green algae; *Spirulina platensis*; Selenium; Iodine; Neutron activation analysis

1. Introduction

The current scientific and technological progress and increasing tempo of everyday human life give rise to civilization diseases. Mental and physical stress and deterioration of environment contribute to metabolic disorder and dysfunction of the immune system, which in turn results in an increasing morbidity rate for cardiovascular, virus, cancer and many other serious diseases.

Changes in the diet, such as limited consumption of natural foodstuff and switching-over to refined food lacking vitamins, mineral components and cellulose while being rich in fat and carbohydrates, have also become serious risk factors for the human organism. These are the obvious reasons why new progressive trends are being extensively developed in modern medicine, pharmacology, and biotechnology and more effective harmless medicaments are being sought for to treat and prevent various diseases.

* Corresponding author. Fax: +7-09621-65085
E-mail address: marina@nf.jinr.ru (M.V. Frontasyeva).

0731-7085/02/$ - see front matter
PII: S0731-7085(02)00199-1

One of such trends in biotechnology is associated with the blue–green microalga *Spirulina platensis*, which has been widely used since the 1990s. The biomass of spirulina and its processing products are employed as feed and food additives in agriculture, food industry, pharmaceutics, perfumery making, medicine, and science.

This wide use is due to its fast growth, non-toxicity, assimilability (85–95%), high protein content (60–70%), well-balanced amino acid composition, richness in vitamins, and a great variety of biologically active agents in appreciable amounts [1,2].

Spirulina is held to work profitably as an immunopotentiator and is characterized by the anticarcinogenic and antiviral effects.

The experts of the World Health Organization have found out that *S. platensis* as a health-improving agent surpasses all so far known food elements and medicaments.

Spirulina is often used before and in the course of drug treatment to remove harmful agents from the organism and to introduce a variety of vital biologically active elements and compounds, which results in normalizing of metabolism and strengthening of the immune system, i.e. favorable conditions for the action of drugs are provided. An example is the use of spirulina to treat children who suffered from the Chernobyl accident [3].

2. Formulation of the problem

Investigations in the field of molecular biology show that most human illnesses are so-called 'molecular' diseases stemming from lack of some elements and compounds in the organism.

One of such elements is selenium. Biological importance of selenium for the human organism was recognized over 40 years ago, and particular progress in comprehending mechanisms of its biological and medical effect has been made in the past 20 years [4]. Selenium is a normal component of some enzymes, proteins and aminoacryl derivatives of nuclei acids. Lately selenium has been found to be incorporated in the 21st amino acid, selenocysteine, which plays a unique part in reading genetic information during synthesis of proteins [5].

Selenium lowering may cause such diseases as cardiomyopathy, cancer, endemic osteoarthropathy, anemia, etc. In China there is a vast area with soils low in selenium, the so-called 'selenium-deficient zone', where these diseases of endemic nature occur [6]. The investigations performed in the United States showed that in areas where the selenium content of cereals is low mortality due to various kinds of cancer is higher than in other areas [7]. Trace elements are known to be play important part in oxygen metabolism. Some of them, e.g. Fe and Cu, form free hydroxyl radicals while others, such as Zn and Se, can reduce the harmful effect of radicals on the organism. Selenium owes this property to its being incorporated in glutathione peroxidase, an antioxidant enzyme protecting cells against peroxidation stress [8,9]. The glutathione peroxidase activity is affected not only by the selenium content but also by the concentrations of vitamin A and vitamin *C* that help to assimilate, transport and utilize selenium in the enzyme. The function of selenium in the organism is closely related to functions of vitamin *E* and beta-carotene. According to some hypotheses, inhibition of carcinogenesis after administering selenium is due to its antioxidant property, impact on tumor metabolism, effect on the endocrine and immune systems, and inhibition of specific enzymes.

Selenium takes part in photochemical reactions related to vision. Glutathione peroxidase, incorporated in retina photoreceptors, affects photosensitivity of the eye [10,11]. Selenium also allows detoxification of the organism by helping to bind some harmful elements, such as As, Cd, Hr, Bi, etc, to high-molecular proteins of blood plasma and to remove them from the organism. In the absence of selenium these elements are bound to a low-molecular protein metallothioneine and settle together with it in kidneys [10,12].

Investigations at the subcellular level show that human cells contain selenium in their nuclei, mitochondria, cytoplasm, microsomes, etc. [6,13,14]. Plenty of selenium is found in mitochondria, which play an important part in processes of electron transport, ATP synthesis, and others.

Variation in its content strongly affect the functions and structure of mitochondria. It is dysfunction of myocardium mitochondria that causes cardiomyopathy (Keshan disease).

It has been found that selenium added to diet in particular doses decreases the cancer hazard, favors treatment of cardiological patients, reduces the acquired immunodeficiency syndrome (AIDS), slows down the aging, etc. [15–19]. On the other hand, a high level of selenium causes toxicosis.

Selenium is used for treatment in various combinations: in some cases 50 μg of selenium are added to food with α-tocopherol (vitamin *E*) and beta-carotene, in other cases it is combined with 26 minerals and vitamins [9]. In [9] treatment of carcinoma of the skin included daily administration of 200 μg of selenium with a food additive (nutrition-21). Selenium is most often used with vitamin *E*, deficiency of which often accompanies selenium deficiency [9,20].

The diverse importance of selenium for the human organism is emphasized in the proceedings of the 7th International Symposium Selenium-2000 (Venice, October 2000), where selenium was called the element of the century. According to the data of the Institute of Nourishment of the Russian Academy of Medical Sciences (Moscow), selenium deficiency is found practically over the whole of Russia. In 1998 a program 'Selenium, Health, Man' was launched in the Russian Federation and selenization of the population is being carried out within its framework.

Another equally important element incorporated in all plants and animals is iodine. It is vitally important for functioning, development and growth of the organism, where it comes with food, water and air. The foodstuffs richest in iodine are milk, vegetables (especially cabbage), eggs and seafood.

Iodine affects metabolism enhancing the oxidation–reduction processes. Iodine deficiency results in dysfunction of the thyroid, less of its hormones thyroxin and triiodothyronine are ejected into blood, which eventually leads to hypothyroidism.

Iodine content of the air strongly depends on proximity of the area to the sea. The sea air can satisfy the daily iodine demand of man (~ 200 μg). On the contrary, the air in the mountain areas is low in iodine, which entails iodine deficiency in the human organism and thus mass thyroid diseases. An example of such an area is mountain Svanetia in Georgia, which shows a pronounced mass trend toward thyroid diseases. The features of these endemic diseases and their prevention methods were well studied in the 1950–1960s [21].

In 1960–1980s the morbidity slightly decreases owing to improved living standards and preventive measures involving consumption of iodinated food products. Later, however, the situation changed for the worse.

After the Chernobyl accident in April 1986, radioactive iodine ^{131}I and other radionuclides were seen to be spreading over the Ukraine, Byelarussia, Georgia and some regions of Russia for 2–3 months. Investigations carried out in 1986 showed elevated concentration of ^{131}I in milk, milk products and vegetables, especially in early cabbage [22,23].

Though the isotope ^{131}I has a relatively short half-life of 8 days, its accumulation in the thyroid leads to serious dysfunction of the gland due to beta and gamma radiation.

Children, whose basic dietary component was milk enriched in radioactive iodine, were particularly badly affected. The investigations showed that the concentration of ^{131}I in thyroid cells of children was 12 times larger than in adults in Georgia over that period [24]. Apart from the radiobiological effect, there can also be the genetic effect with its far-reaching consequences.

Unfavourable environmental conditions, discharge of radioactive materials into the air during accidents, lowering of living standards have aggravated the situation both on the post-Soviet territories and in the whole world. In Russia about 70% of the population are suffering iodine deficiency in varying degrees.

Recently the symptoms and results of iodine deficiency have been more thoroughly studied on the emotional level (irritability, memory impairment, sleepiness, etc.), cardiological level (atherosclerosis, arrhythmia, deformation of vascular walls, etc.), immunodeficient level (susceptibility to infections and colds). The intelligence quotient (IQ) is found to be directly related to the iodine concentration in the organism.

It was found out that mental retardation of 43 million people in the world is due to iodine deficiency. Every year 100,000 children suffering cretinism because of iodine deficiency are born. Therefore, elimination of diseases caused by iodine deficiency is one of UN priorities in the field of human health.

The experience of using iodinated salt to prevent iodine deficiency in the United States, Switzerland, and other countries showed that iodine excess results in such a thyroid disease as iodine-induced hyperthyroidism. The investigations showed that only biotransformed iodine synthesized by protein molecules is assimilated by the organism in the required amount, neither less nor more.

The ability to biotransform and endogenously add the desired elements (selenium, iodine, etc.) producing complexes easily assimilated by a human organism is a distinctive feature of *S. platensis*. Being a living organism, spirulina accumulates elements strictly as much as is necessary for the organism. Spirulina-based preparations contain a complex of biologically active agents and produce both therapeutic and health-improving effect. For example, spirulina-based selenium preparations will also contain tocopherol (vitamin *E*) and beta-carotene that are necessary for treatment of some diseases [9,19].

All the aforesaid has led to a hypothesis that it would be reasonable to investigate *S. platensis* as a matrix for production of Se- and I-containing drugs to treat various illnesses. During cultivation of spirulina selenium is built into organic molecules of such compounds as methionine, cysteine, cystine, as well as into protein molecules, etc.

To verify the hypothesis, it was necessary

- to determine the background level of concentrations of elements present in the spirulina biomass;
- to study the dynamics of selenium accumulation in the spirulina matrix at different selenium compound loading of the nutrient medium;
- to study, with mercury loading as an example, the extent to which toxic elements are assimilated by the spirulina biomass;
- to study the influence of selenium loading of the nutrient medium on the growth rate of *S. platensis* and the quality of its biomass;
- to develop, with iodine as an example, a technology for production of treatment and prophylactic drugs (pills) on the basis of *S. platensis* biomass.

To develop new pharmaceuticals the precise analytical control at all technological stages is of great importance. Neutron activation analysis was chosen for this purpose as the most suitable method for these investigations. The advantages of the instrumental neutron activation analysis in its epithermal-neutron version (ENAA) for studying the many-element composition of biological samples were shown earlier [25]. Therefore, the composition of *S. platensis* was studied by ENAA widely used at the pulsed fast reactor IBR-2 (JINR FLNP, Dubna) with a very high epithermal-to-thermal neutron ratio.

3. Materials and methods

3.1. Conditions for cultivation of S. platensis

Spirulina grows well in a standard alkaline mineral nutrient at a temperature of 30–34 °C, pH 8.5–11, under sodium lamp light. The maximum growth of cells occurs on the 4–5th day of cultivation. Cultivation was carried out in a 20-l bioreactor. The nutrient was made with distilled water to which inorganic components were added [26].

3.2. Sample preparation

After cultivation the spirulina cell mass was separated from the nutrient, washed with distilled water three times and centrifuged. The resulting wet biomass was lyophilically dried in a adsorption-condensation lyophilizer of a unique design developed by us according to the technique described in [27,28]. The native dry biomass was made into small pellets of various diameters and thickness by means of a special titanium mould.

3.3. Experiment

1. To study background concentrations of various elements in the *S. platensis* biomass, cultivation was carried out in a standard nutrient with distilled water.
2. To study selenium accumulation dynamics in the *S. platensis* biomass, cultivation was carried out in a nutrient with selenium loading (selenious acid H_2SeO_3, chemically pure grade), selenium concentrations ranging from 0.5 to 15 mg/l.
3. To study the possibility of toxic elements being incorporated in the spirulina biomass, cultivation was carried out in a nutrient with mercury loading (mercury nitrate $Hg(NO_3)_2 \cdot 0.5H_2O$, pure grade), concentrations of mercury (used for a toxic element) ranging from 0.0033 to 33 μg/l.
4. To develop the technology for production of iodine-containing treatment and prophylactic pills on the basis of the *S. platensis* biomass, cultivation was carried out in a nutrient with potassium iodide loading (KI, highly pure grade), KI concentrations ranging from 10^{-8} to10^{-4} g/l. The experimental conditions were chosen from the following considerations. The daily iodine demand for an adult is known to be some 200 μg. A normal daily iodine loss through secretion is some 100–200 μg. Therefore, a treatment pill should contain about 200–500 μg of iodine and a prophylactic pill should contain of the order of 100–200 μg of iodine.
5. To examine the dependence of the *S. platensis* cells growth rate on the nutrient medium loading with selenium compounds H_2SeO_3, cultivation was performed at different concentrations (1–100 mg/l) introduced to the nutrient medium in the first day of cultivation ('dose–effect').

3.4. Analysis

Spirulina samples from the first four experiments enlisted above were subjected to multi-elements instrumental neutron activation analysis. Samples weighing some 0.5 g were packed in aluminium foil to determine long-lived isotopes and in polyethylene to determine short-lived isotopes. The characteristics of irradiation channels connected with the pneumatic transport system of the IBR-2 reactor at JINR FLNP are listed in Table 1.

To determine long-lived isotopes, irradiation channel Ch1 was used. The samples were irradiated for 5 days, repacked and measured two times, after being kept for 4 and 20 days. The measurement time varied from 1.5 to 10 h. To determine short-lived Mg, Al, Cl, Ca, V, Mn and I isotopes, irradiation channel Ch2 was used. Samples were irradiated for 3 minutes and measured two times, after being kept for 3–5 and 20 min. The measurement time was 5–8 and 20 min respectively. The gamma spectra of induced activity were recorded with a large-volume super-pure germanium detector with a resolution of 1.96 keV for the ^{60}Co 1332.4-keV gamma line and the recording efficiency of 30% with respect to the $3 \times 3''$ NaI detector efficiency for the same line.

Table 1
Basic characteristics of the irradiation channels at the IBR-2 reactor of the JINR FLNP [29]

Irradiation channel	3Neutron flux density (n/cm^2 s) 10^{12}			T (°C)	Channel diameter (mm)	Channel length (mm)
	Thermal	Resonance	Fast			
Ch1	Cd screen	3.31	4.32	70	28	260
Ch2	1.23	2.96	4.10	60	28	260

Table 2
Comparison of the recommended values and the NAA results for the synthetic many-element standards SSB-1 and SSB-2

Elements	Introduced amount of element [32]	NAA-determined amount of element [32]	ENAA-determined amount of element (present paper)
SSB-1			
Se	2.84	2.80 ± 0.07	2.26 ± 0.33
Cr	1.80	1.78 ± 0.05	1.85 ± 0.20
Au	0.028	0.026 ± 0.0006	0.034 ± 0.0008
Sb	0.47	0.45 ± 0.01	0.44 ± 0.09
Ag	1.89	1.72 ± 0.05	1.92 ± 0.12
Rb	9.45	9.05 ± 0.26	9.12 ± 1.09
Fe	284	296 ± 6	315 ± 40
Zn	18.9	19.5 ± 0.5	22.05 ± 3.52
Co	0.66	0.63 ± 0.05	0.75 ± 0.07
SSB-2			
Ca	5210	3740 ± 160	5400 ± 500
Ba	10.3	10.6 ± 0.3	10.2 ± 1.2
Hg	0.50	0.10 ± 0.01	0.14 ± 0.04
Sn	30.8	24.0 ± 1.9	28.0 ± 4.0
Br	20.8	20.7 ± 0.6	19.4 ± 2
Cs	0.50	0.48 ± 0.01	0.4 ± 0.1
Ni	10.3	9.5 ± 0.6	9.83 ± 1
Sc	37	37.2 ± 0.8	37.8 ± 2
Na	3070	3660 ± 590	3500 ± 320

The data were processed and the element concentrations were determined with certified reference items normally used at the laboratory [30].

Since selenium and mercury are volatile elements, the irradiation temperature is very important. The critical temperature for correct determination of Se and Hg in biological samples is known to be 90–100 °C [31]. The irradiation temperature in channels Ch1 and Ch2 did not exceed 60–70 °C [29]. Yet, insignificant Se and Hg loss is not excluded. The contribution from the ^{203}Hg line was taken into account when ^{75}Se and ^{182}Ta were determined by the 279.1-keV gamma line.

The neutron multiplier PS-1 with a pneumatic transport system at the Institute of Physics of the Georgian Academy of Sciences was used as a neutron source for INAA of iodine-enriched spirulina samples. With the neutron flux density 2×10^6 n/(cm^2 s), $T_{irr} = 600$ s, $T_{stay} = 60$ s and $T_{meas} = 410$ s, the ^{128}I 442.9-keV gamma line (16.9%) was recorded with a sensitivity of 2×10^3 decays/(s mg) of iodine, which is sufficient for analysis of iodine-enriched spirulina samples.

3.5. Analytical quality control

Three certified standards, IAEA Lichen-336, IAEA Bottom Sediments SDM-2T and Nordic Moss DK-1 were used to control the quality of analytical measurements. In addition, synthetic many-element standards SSB-1 and SSB-2 for NAA of biological samples [32], locally developed on the basis of phenol-formaldehyde resin, were used. Table 2 shows good agreement of the results with the recommended values. On the whole the analysis accuracy was 10–15%. The discrepancy between the introduced and determined amounts of Hg (columns 2 and 3) is due to evaporation of mercury during the phenol-formaldehyde resin synthesis procedure [32].

4. Discussion of results

ENAA was used to determine the background level of elemental concentrations in Spirulina biomass. A total of 31 macro-, micro- and trace elements were determined from the first experi-

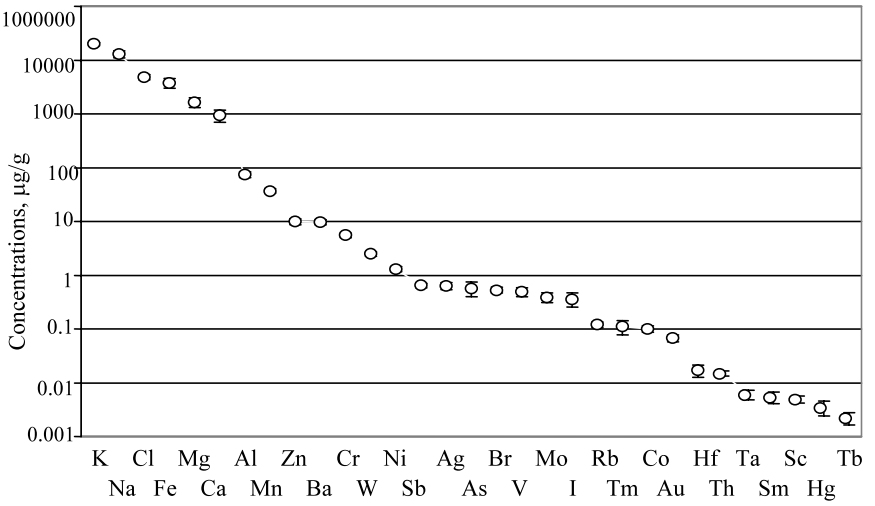

Fig. 1. Background concentrations of macro- and microelements in the spirulina biomass.

ment (cultivated in a standard nutrient with distilled water). The results are shown in Fig. 1, where the elements are given in order of descending concentration within eight orders of magnitude from macroelements to ultra trace elements. In the future this set of elements will be extended by including data on Pb, Cd, Cu etc. that can be obtained by other analytical methods, such as atomic absorption spectrometry (AAS) and induction-coupled plasma mass spectrometry. The NAA results for K, Na, Ca, Mg, Mn and Zn are in good agreement with the 1996 AAS results [33]. The iron concentration found by ENAA is twice as high as the concentration found by ASS, which is likely to be due to incomplete dissolving of iron compounds in the course of sample preparation for AAS.

The NAA results of experiments with selenium, iodine and mercury loading are summed up in Table 3 and are displayed in Fig. 2. The concentration dependence of selenium in spirulina biomass is well approximated by the six-order polynomial with $R^2 = 0.99$. The increase of selenium accumulation is observed with maximum in the range with 13 mg/l. For iodine and mercury approximation is described by polynomial of the second-order.

Extensive incorporation of mercury begins, when the Hg concentration in the nutrient is about 10 μg/l.

Table 3
Dependence of Se, I and Hg accumulation in the *S. platensis* biomass on loading of the nutrient with these elements in various concentrations

Sample	Loading of nutrient, μg/l	*S. platensis*, μg/g
Se control[a]	–	0.69 ± 0.09
1+Se	500	8.27 ± 0.65
2+Se	1000	12.2 ± 0.65
3+Se	2000	26.7 ± 1.39
4+Se	3000	34.1 ± 1.57
5+Se	4000	30.2 ± 1.51
6+Se	5000	44.1 ± 2.03
7+Se	6000	52.9 ± 2.49
8+Se	8000	46.9 ± 2.16
9+Se	10 000	78.5 ± 3.61
10+Se	12 000	136 ± 6.26
11+Se	15 000	85.5 ± 3.93
I control[a]	–	0.3
1+ I	170	0.24
2+I	250	0.42
3+I	500	2.00
Hg control[a]	–	<0.050
1+Hg	0.0033	0.09 ± 0.05
2+Hg	0.033	0.38 ± 0.11
3+Hg	0.33	0.24 ± 0.07
4+Hg	3.3	0.49 ± 0.15
5+Hg	33	3.9 ± 1.2

[a] Background Se, I and Hg content of the spirulina biomass.

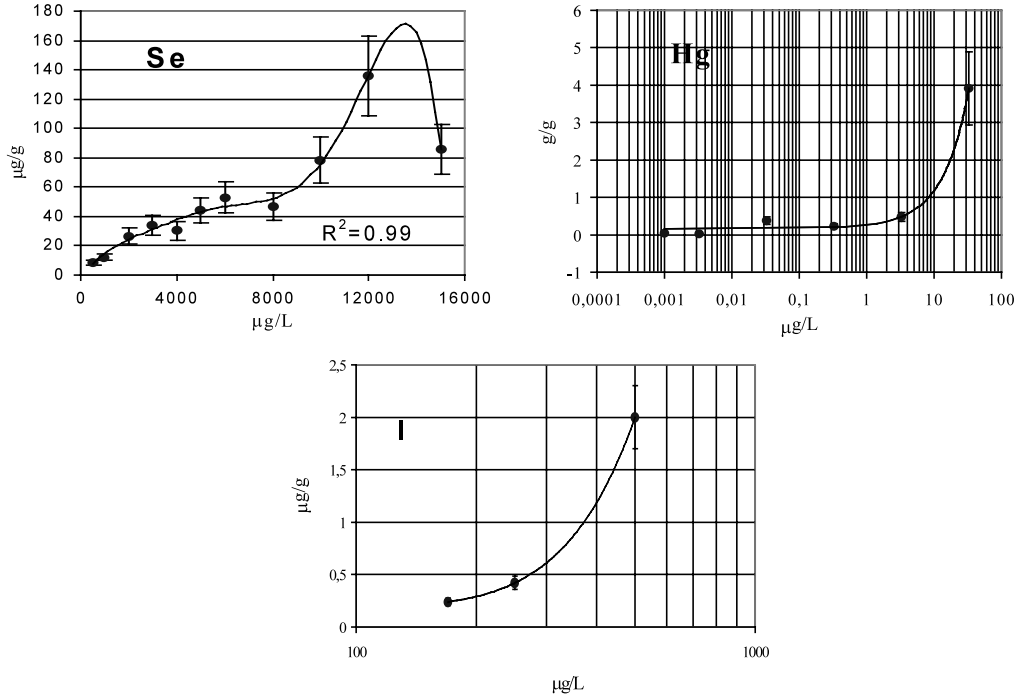

Fig. 2. Concentration of selenium, iodine and mercury in the spirulina biomass as a function of their concentration in the nutrient.

It can be assumed that in spirulina selenium is bound to the amino acid cystine because its concentration in the spirulina biomass is about 1% (see http://www.spirulina.com/SPBNution.html). Possible formation of Se-cystine complexes and their anticarcinogenic role is discussed in [4].

Cell cultivation is accompanied by synthesis of nucleic acids, proteins, hydrocarbons, pigment, lipids and other macromolecular structures. Biomolecular interactions with various chemical elements can be of different nature and character, from weak bonding with free energy ranging between 1 and 7 kcal/mol to strong covalent bonding with energy ranging from -50 to -110 kcal/mol.

According to our estimation, the elements determined in the present paper by INAA and ENAA show weak bonding, like Van der Waals (1–2 kcal/mol) or hydrogen (3–7 kcal/mol) one. It is well known that hydrogen bonding plays an important part in biomolecular interactions. Therefore, in the future it is reasonable to study the nature of the binding energy of double and triple complexes like Me–DNA, Me–protein and Me–DNA-protein.

The technology for production of treatment and prophylactic preparations was being developed with iodinated *S. platensis* biomass on the basis of the INAA data obtained at the neutron multiplier (Table 4).

The iodine enrichment coefficient R was defined as a ratio between the iodine concentration in the spirulina biomass and the iodine concentration in the nutrient and served as an initial technological parameter governing the iodine dosage in treatment pills and the choice of the pill mass.

Iodinated pills were made by the proposed technology with *S. platensis* used as a matrix. The characteristics and marking of the pills are given in Table 5.

Pills marked as I-100 and I-200 are intended for preventing diseases caused by morning iodine deficiency and treating them at early stages, while I-400 and I-500 pills are used in particularly severe cases.

Table 4
Initial data for production of pills on the basis of the *S. platensis* biomass

Iodine content of nutrient, mg/l	Yield of lyophilised biomass, g/l	Iodine concentration in biomass, mg/l	Iodine enrichment coefficient, *R* (%)
170	0.45	0.24	0.14
250	0.30	0.42	0.17
500	0.80	2.00	0.40

Table 5
Composition and shape of iodinated pills based on the *S. platensis* biomass

Marking	Diameter of pill (mm)	Biomass of pill (g)	Iodine content (μg)
I-100	5	0.5	100
I-200	5	0.5	200
I-400	10	1.0	400
I-500	10	1.0	500

In the course of cultivation spirulina cells may assimilate some toxic elements, like Hg, As, Cr, Cd, Pb, etc., present in the nutrient as impurity. Table 6 presents their background concentrations in the spirulina biomass produced with reagents of the given grade.

As is evident from the above results, concentrations of toxic elements in the *S. platensis* biomass are in the order of μg/g. Trace amounts of these elements appear in chemical reagents used to prepare a nutrient. Therefore, highly pure chemicals must be used to produce biomass for pharmaceutical purposes.

On the other hand, the US data on permissible doses of various elements for a human organism (see http://www.spirulina.com/SPBNnutrition.html) show that our results obtained with the reagents of the chemically pure and pure grade do not exceed the permissible level.

The results obtained in experiments 'dose–effect' are shown in Fig. 3. As follows from this figure, when the Se concentration is 50–100 mg/l, the effect of cells growth rate elimination becomes significant after 6 days of cultivation, and on the 12th day the amount of spirulina biomass reaches 25–30% of that grown without nutritional loading. At low concentration of selenium loading the effect of cells elimination is insignificant.

5. Conclusions

1. Composition of the blue–green microalga *S. platensis* biomass is studied. Concentrations of 31 macro- and microelements are found in a wide range by the ENAA method. It is shown that the spirulina biomass cultivated as proposed does not incorporate toxic elements in concentrations higher than permissible and can be used as a matrix for production of drugs.
2. Target-oriented introduction of selenium and iodine in the spirulina biomass is shown to be possible during cultivation. Polynomial relationship between accumulation of selenium

Table 6
Background concentrations of some toxic elements in the *S. platensis* biomass

Element	Dry mass concentration, μg/g
Hg	3–5[a]
As	2–3[a]
Cr	3–4[a]
Pb	3[b]
Cd	0.2[b]

[a] NAA data obtained at the IBR-2 reactor in Dubna [34].
[b] data from [35,36].

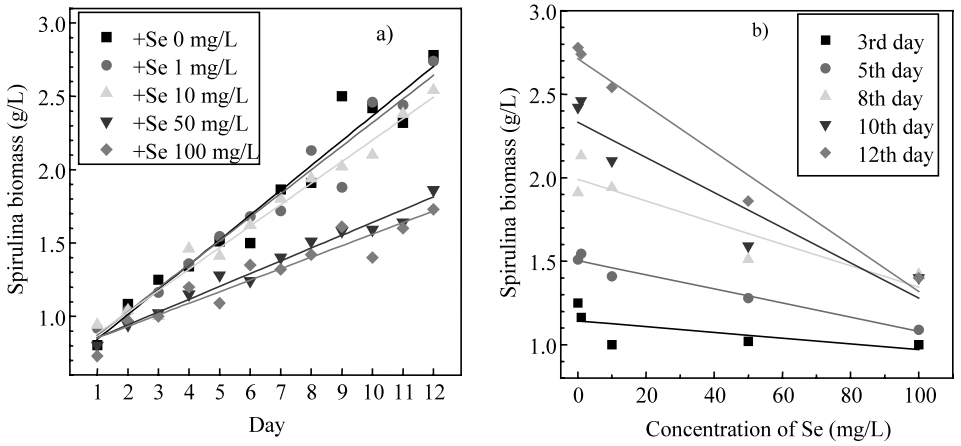

Fig. 3. Dependence of *S. platensis*. biomass growth on Se concentration in the nutrient medium.

and iodine in the spirulina biomass and their concentration in the nutrient is found.

3. It is demonstrated with mercury that the spirulina biomass can accumulate toxic elements.
4. It is demonstrated with mercury loading of the nutrient that the dangerous concentration of toxic elements can be preliminarily determined in the course of drug production. It is found out that highly pure reagents are desirable for cultivation of spirulina biomass for pharmaceutical purposes.
5. Important technological parameters for production of treatment-and-prophylactic iodinated pills are established on the basis of NAA of the *S. platensis* biomass.
6. The chosen conditions of spirulina biomass cultivation with loading with the given elements provides their endogenic inclusion in Spirulina biocomplexes without deteriorating its natural features.

Acknowledgements

The authors are very grateful to Professor A. Chatt (Dalhousie University, Canada) and Professor E. Steinnes (Norwegian University of Science and Technology, Trondheim) for helpful discussions. The present investigation was supported by the International Atomic Energy Agency, project IAEA-11528/Regular Budget Fund (RBF).

References

[1] D. Fox, Health Benefits of Spirulina, in Spirulina, Algae of Life, Bulletin No. 12, Institute of Oceanography, Monaco April 1993.
[2] A. Belay, Y. Ota, K. Miyakawa, H. Shimamatsu, Current Knowledge on Potential Health Benefits of Spirulina, J. Appl. Phycol. 5 (1993) 235–241.
[3] L. Evets et al., Means to normalize the levels of immunoglobuline E using the food supplements Spirulina, 1994, Grodnenski State Medical Univ. Russian Federation Committee of Patents and Trade, Patent (19)RU(11) 2005486, Russia January 15, 1994.
[4] H. Foster, Health, Disease and the Environment, Belhaven Press, London, 1992.
[5] J.F. Atkins, R.F. Gestland, The twenty-first amino acid, Nature 407 (2000) 463–464.
[6] C. Chen, P. Zhang, X. Hu, Z. Che, Subcellular distribution of selenium and Se-containing proteins in human liver, Biochem. Biophys. Acta 1427 (1999) 205–215.
[7] L.C. Clark, K.P. Cantor, W.H. Allaway, Selenium in forage crops and cancer mortality in US countries, Arch. Environ. Health 46 (1991) 37–42.
[8] C. Chen, B. Chen, J. Zhou, H. Xu, Study of the relationship of reactive oxygen species and biological dose-responsive curve of selenium, Trace Elem. Electrolytes 14 (4) (1997) 197–201.
[9] L.C. Clark, G.F. Combs, B.W. Turnbull, et al., Effect of selenium supplementation for cancer prevention in patients with carcinoma of the skin, J. Am. Med. Assoc. 276 (24) (1996) 1957–1963.
[10] Y.A. Petrovich, R.P. Podorozhnaya, Selenoenzimes and other selenoproteides, their biological role, Uspekhi Sovremennoi biologii 91 (1) (1981) 127–144 In Russian.
[11] Zh.I. Abramova, G.I. Oksengendler, Man and antioxidants, L., Nauka, 1985 in Russian.

[12] L.A. Daniel, Selenium metabolism and bioavailability, Biol. Trace Elem. Res. 54 (1996) 185–199.
[13] L.K. Tkeshelashvili, I.S. Mepisashvili, L.M. Mosulishvili, N.E. Kuchava, Neutron activation analysis of heavy metals in sub-cellular components, in: Radiation Investigations, vol. II, Tbilisi, Metzniereba, 1975, pp. 25–32 (in Russian).
[14] C. Chen, P. Zhang, X. Hu, Z. Che, Investigation of selenium distribution in subcellular fraction of human liver by neutron activation analysis, Biol. Trace Elem. Res. 71-72 (1999) 131–138.
[15] G.F. Combs Jr, Chemopreventive mechanisms of selenium, Med. Klin. 94 (3) (1999) 18–24.
[16] M. Navarro-Alarcon, H.L. de la Serrana, V. Perez-Valero, C. Lopez-Martinez, Serum selenium levels as indicators of body status in cancer patients and their relationship with other nutritional and biochemical markers, Sci. Total Environ. 212 (2-3) (1998) 195–202.
[17] K. Schumacher, Effect of selenium on the side effect profile of adjuvant chemotherapy/radiotherapy in patients with breast carcinoma. Design for a clinical study, Med. Klin. 94 (3) (1999) 45–48.
[18] R. Ebert-Duming, J. Seufert, D. Schneider, J. Kohrel, N. Schutze, F. Jacob, Expression of selenoproteins in monocytes and macrophages-implications for the immune system, Med. Klin. 94 (3) (1999) 29–34.
[19] D.H. Holben, A.M. Smith, The diverse role of selenium within selenoproteins: a review, J. Am. Diet. Assoc. 99 (7) (1999) 836–843.
[20] J.E.van Vleet, V.J. Ferrans, Etiological factors and pathology alteration in selenium-vitamin E deficiency and excess in animals and humans, Biol. Trace Elem. Res. 33 (1992) 1–21.
[21] A.I. Voinar, Trace elements in Nature, M., (1962) 74–92 (in Russian).
[22] L.M. Mosulishvili, N.M. Katamadze, N.I. Shoniya, E.I. Ginturi, Kinetic regularities of change in the concentration of radionuclides in the georgian tea content, Med. Radiol. 1 (1990) 42–45.
[23] L.M. Mosulishvili, N.I. Shoniya, N.M. Katamadze, E.N. Ginturi, Environmental radionuclide distribution in Georgia after the Chernobyl catastrophe, J. Anal. Chem. 49 (1) (1994) 135–139 in Russian.
[24] N. Katamadze, N. Kuchava, L. Mosulishvili, M. Tsitskishvili, Evaluation of thyroid gland irradiation dose induced by Chernobyl radiation for Tbilisi region population, Bull. Georg. AS 157 (2) (1998) 213–216.
[25] M.V. Frontasyeva, E. Steinnes, Epithermal neutron activation analysis for studying the environment, Proc. Int. Symposium on Harmonization of Health Related Environmental Mesurements Using Nuclear and Isotopic Techniques (Hyderabad, India, 4–7 November, 1996), IAEA 1997, 301–311.
[26] L. Mosulishvili, A. Belokobilsky, E. Gelagutashvili, A. Rcheulishvili, N. Tsibakhashvili, The study of the mechanism of cadmium accumulation during the cultivation of *Spirulina platensis*, Proc. Georg. Acad. Sci. Biol. Ser.1 23 (1–6) (1997) 105–113.
[27] L.M. Mosulishvili, V.S. Nadareishvili, N.E. Kharabadze, A.I. Belokobilsky, Facility for lyophilization of biological preparations, Patent USSR N779765, Bull. 42 (1980).
[28] L. Mosulishvili, N. Kharabadze, A. Belokobilsky, A. Gudushauri, Application of the absorbtion-condensation method of lyophilization in the activation analysis of biological materials, Bull. Georg. AS 98 (3) (1980) 685–688.
[29] M.V. Frontasyeva, S.S. Pavlov, Analytical investigations at the IBR-2 reactor in Dubna, JINR Preprint E14-2000-177, Dubna, 2000.
[30] T.M. Ostrovnaya, L.S. Nefedyeva, V.M. Nazarov., S.B. Borzakov L.P. Strelkova, Software for INAA on the basis of relative and absolute methods using nuclear data base, in: Activation analysis in environment protection, D-14-93-325, Dubna, 1993, pp. 319–326.
[31] L.M. Mosulishvili, N.E. Kuchava, N.I. Shonia, Investigation of the termostability of Synthetic Standards for instrumental neutron activation analysis of biological samples, Proc. Georg. AS Biol. Ser. 107 (2) (1982) 501–504.
[32] L.M. Mosulishvili, V.Yu. Dundua, N.E. Kharabadze, E.Yu. Efremova, N.V. Chikhladze, Synthetic multielement standard samples for serial neutron activation analysis of biological materials, J. Radioanal. Nucl. Chem. 1 (1984) 13–19.
[33] A. Belokobilsky, M. Gelashvili, L. Mosulishvili, A. Rcheulishvili, A. Khizanishvili, Investigation of the change of *spirulina platensis* phisical-chemical properties in the growth dynamics, Proc. Georg. AS Biol. Ser. 22 (1-6) (1996) 104–109.
[34] L.M. Mosulishvili, Ye.I. Kirkesali, A.I. Belokobylsky, A.I. Khizanishvili, M.V. Frontasyeva, S.F. Gundorina, C.D. Oprea, Epithermal neutron activation analysis of blue–green algae *Spirulina platensis* as a matrix for selenium-containing pharmaceuticals, Journal of Radioanalytical and Nuclear Chemistry, 252, 1 (2002) 15–20.
[35] A. Belokobilsky, N. Tsibachashvili, A. Rcheulishvili, L. Mosulishvili, Accumulation of Cd(II) in C-phycocyanin from *Spirulina platensis*, Bull. Georg. AS 159 (1) (1999) 140–143.
[36] J.J. Ortega Calvo, C. Mazuelos, B. Hermosin, C. Saiz-Jimenez, Chemical composition of Spirulina and eucaryotic algae food products marketed in Spain, J. Appl. Phys. 5 (1993) 425–435.