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EFFECT OF THE ADDITION OF ZINC AND SELENIUM IONS ON THE STABILITY OF THE BIOLASOL LIQUID USED FOR PERFUSION, REPERFUSION AND PRESERVATION OF PARENCHYMAL ORGANS OF THE ABDOMINAL CAVITY*

Aneta Ostróżka-Cieślik[1], Barbara Dolińska[2], Artur Caban[3], Florian Ryszka[4]

[1,2] Department of Applied Pharmacy
School of Pharmacy with the Division of Laboratory Medicine in Sosnowiec
Medical University of Silesia
[3] Department of General, Vascular and Transplant Surgery
Medical University of Silesia, School of Medicine, Katowice
[4]Pharmaceutical Research and Production Plant "Biochefa", Sosnowiec

Abstract

The Biolasol® liquid is an innovative solution used for perfusion, reperfusion and preservation of parenchymal organs of the abdominal cavity. Substances in the liquid prevent cellular oedema and help to maintain a proper water/mineral as well as acid/base balance in the intracellular environment. They also minimize free-radical injuries and ensure the integrity of the cellular membrane structure. The Biolasol® liquid has been shown to be much more efficient than the HTK liquid in the preservation of kidneys. The Biolasol® liquid containing 0.5 mM of vitamin C has been modified by adding ions of Se(IV), Zn(II), and their effect on the stability of the solution was examined. An accelerated aging test was applied to test the liquid stability. The test, based on the laws of chemical kinetics, was conducted at four temperatures at a 10°C step, that is: 50°C±0.05, 60°C±0.05, 70°C±0.05 and 80°C±0.05. The relative humidity equalled 75% of RH and the duartion of the test was 40 days. In order to determine the stability of the tested solutions, the Arrhenius Dependence equation was used, applied to the effect of temperature on the glucose decomposition reaction rate: $\ln k = \ln A - (E_a/RT)$. The results indicate that the addition of zinc decreases the stability of the liquid by 30.5%, while the addition of selenium

dr n.farm. Aneta Ostróżka-Cieślik, Department of Applied Pharmacy, School of Pharmacy with the Division of Laboratory Medicine in Sosnowiec, Medical University of Silesia, Kasztanowa 3, 41-200 Sosnowiec, Poland, aostrozka@sum.edu.pl

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prolongs the stability by 8.21%. This is explained by the synergism of action of vitamin C and Se^{4+} antioxidant in the tested liquid. Zinc ions present in the solution increase the glucose decomposition reaction rate.

Key words: Biolasol, zinc, selenium, accelerated aging test.

INTRODUCTION

The Biolasol® liquid is an innovative solution used for perfusion, reperfusion and preservation of parenchymal organs of the abdominal cavity (Ryszka et al. 2005). It demonstrates high efficiency in the prevention of ischaemia injuries of organs and injuries resulting from exposure to low temperatures. Substances in the liquid prevent cellular oedema and help to maintain a proper water/mineral and acid/base balance in the intracellular environment. They also minimize free-radical injuries and ensure the integrity of the cellular membrane structure. The Biolasol® liquid has been shown to be much more efficient than the HTK liquid in the preservation of kidneys. It has been found that Biolasol® ensures proper homeostasis of the organ in the preservation period and prevents the occurrence of metabolic acidosis by keeping the pH within the standard 6.8-7.7 (Dolińska et al. 2012*a*) range.

The Biolasol® liquid was modified using two antioxidants: Se(IV), Zn(II), and their effect on the stability of the solution was examined. Selenium is an ingredient of tetraiodothyronine 5 deiodinase, an enzyme catalyzing the deiodination of thyroxine in the liver and kidneys (Sher 2001). Oral substitution with selenium in the form of Na_2SeO_3 in the amount of 200 µg/day increases the production of cytotoxic T cells and the immune system cells (Hardy and Hardy 2004). This element has been found to protect parenchymal organs in the period of ischaemia and reperfusion. Selenium, as the main component of glutathione peroxidase GSH-Px, protects cells against oxidation stress. It also reduces lipid peroxidation, while demonstrating protective action towards DNA (Zapletal et al. 2001, Karayaylali et al. 2004). In periods of ischaemia and reperfusion, selenium shows cardio-protective effects and, if added to the HTK liquid, it stimulates the proper functioning of kidneys after transplantation (Venardos et al. 2004, Tanguy et al. 1998, Poltronieri et al. 1992). Selenium, as an element of the Euro-Collins liquid, ensures an increased protection of lungs in the ischaemia and reperfusion period (Treska et al. 2003, Soncul et al. 1994).

Zinc protects sulfhydryl groups in proteins against oxidation. It is incorporated in superoxide dismutase (CuZn-SOD) and takes part in the elimination of superoxide free radicals. Its high activity in the removal of singlet oxygen has also been verified. The element participates in the biosynthesis of RNA and DNA, and stimulates the proper functioning of cellular membranes. It is the only metal found in enzymes of all classes (Osredkar, Sustar

2011, Powell 2000). Zinc serves to protect the liver in the period of cold ischaemia by inducing the action of heat-shock proteins (Cheng et al. 2002). It has been found that zinc stimulates the proper activity of antioxidant enzymes in the liver in rats with induced protein deficiency (Sidhu et al. 2004). Zinc is also hepatoprotective if the liver becomes exposed to the toxic effects of organophosphorus compounds (Goel et al. 2000). Its protective effect on the kidneys and the heart in periods of ischaemia and reperfusion (Ogawa, Mimura 1999, Chanoit et al. 2008, Karagulova et al. 2007) has been demonstrated.

Zinc ions have been added to St. Thomas' Hospital No. 2 liquid (Plegisol), and their effect on isolated rat hearts has been examined. Consequently, zinc has been found to show to contribute to the maintenance of a proper cardiac activity, hence it may be applied in cardioplegic solutions (Powell et al. 1995). Analyses of the effect of zinc histidinate $Zn(Hs)_2$ in a period of ischaemia and reperfusion on the domestic pig's heart have been performed in an artificial system of extracorporeal circulation, i.e. in a heart-lung apparatus. St. Thomas Hospital No. 2 liquid was used, and the subsequent series of haemodynamic tests confirmed the positive impact of zinc on the myocardial activity (Powell et al. 1997).

In rats with induced diabetes in rats, simultaneous administration of vitamin C and zinc regulates the glucose levels in blood and decreases the concentration of malondialdehyde (MDA), an indicator of lipid peroxidation (Dawud et al. 2012). Administration of sodium selenite and zinc gluconate to patients during haemodialysis, on the other hand, minimizes oxidation injuries (Richard et al. 1993).

MATERIAL AND METHODS

The Biolasol® liquid (produced by the Biochefa Pharmaceutical Research and Production Plant) containing 0.5 mM of vitamin C has been modified by adding ions of zinc and selenium. Zinc acetate [$Zn(CH_3COO)_2$] (produced by POCh S.A Gliwice, Poland) and sodium selenite [Na_2SeO_3] (Sigma-Aldrich, USA) were used for analyses. The tested liquids were prepared in an adequately equipped room, meeting the requirements of GMP (Good Manufacturing Practice), in line with the binding regulations and procedures. Glucose in the liquids was determined using a quantitative glucose determination kit (manufactured by Pointe Scientific Inc. *Oxyglucose* – Catalogue No. G7521). Analytical grade reagents were applied.

A climate chamber and a laboratory incubator (manufactured by POL-EKO-aparatura Sp.j.) were used for the measurement of stability.

Liquid stabilit testing

An accelerated aging test was applied to test liquid stability. The test, based on the laws of chemical kinetics, was conducted at four temperatures at a 10°C step, that is: 50°C±0.05 (323 K), 60°C±0.05 (333 K) 70°C±0.05 (343 K) and 80°C±0.05 (353 K). The relative humidity equalled 75% of RH and the duration of the test was 40 days. The Biolasol® liquid is thermolabile due to its active ingredient, glucose, which is temperature-sensitive. In order to determine the stability of the tested solutions, the Arrhenius Dependence equation was used, determining the effect of temperature on the glucose decomposition reaction rate:

$$\ln k = \ln A - \frac{E_a}{RT},$$

where: k – rate constant, A – frequency coefficient, E_a – activation energy, R – universal gas constant (83.1 hPa x dm3/ mol x K), T – thermodynamic temperature (K).

Plots presenting the dependence of lnk on the inverse absolute temperature have been prepared, a step normally taken for estimation of the impact of temperature on equilibrium reactions (Ostróżka-Cieślik et al. 2009, Mady et al. 2010). Based on the charts, activation energy (E_a) and the frequency factor (A) were established. The liquids for the perfusion and preservation of organs should be kept at appropriately low temperatures, and thus their stability has been established at T = 5°C using the following dependence:

$$t_{90}=0.1053 \ / \ k_{278}.$$

RESULTS AND DISCUSSION

Based on the results, it has been verified that glucose decomposition at all the test temperatures proceeded in line with the kinetics of the first order reaction. Figure 1 illustrates the ln k = f(1/T) dependence.

Table 1 includes the calculated kinetic parameters: the constants of decomposition rates at respective temperatures (k), activation energy (E_a), frequency factor (A) and the established stability of the liquids at 5°C. The results obtained at each of the temperatures have been presented as means of 5 results (k) of independent test repetitions. The data analysis has been performed using the Statistica 10 software and the Stability Appraisal Suite by StatSoft Poland.

The purpose of ascorbic acid in the Biolasol® liquid is to protect a target organ against free oxyradicals, thus increasing survival rates of transplanted

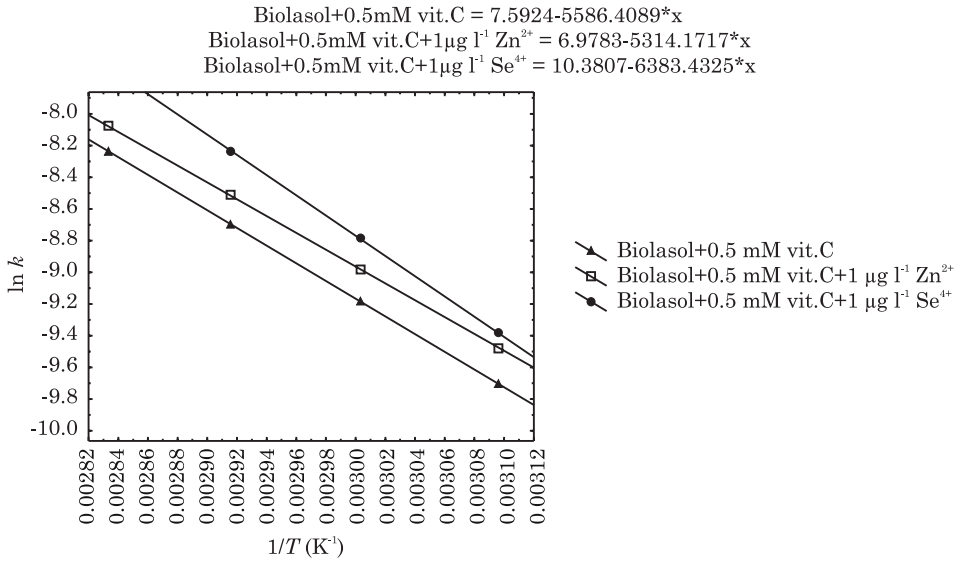

Fig.1. The Arrhenius plot of the glucose decomposition reaction in the Biolasol® solution modified by the addition of vitamin C, Se^{4+} and Zn^{2+}

Table 1

Kinetic parameters of the glucose decomposition reaction as established for the Biolasol solution modified by the addition of vitamin C, Se^{4+} and Zn^{2+}

Kinetic parameters	Biolasol® +0.5 mM vit.C	Biolasol® +0.5 mM vit.C +1 µg l^{-1} Zn^{2+}	Biolasol® +0.5 mM vit.C +1 µg l^{-1} Se^{4+}
$k_{50°C}$ 10^{-5} (h^{-1})	6.102	7.68	8.41
$k_{60°C}$ 10^{-5} (h^{-1})	10.280	12.56	15.29
$k_{70°C}$ 10^{-5} (h^{-1})	16.779	20.14	26.53
$k_{80°C}$ 10^{-5} (h^{-1})	26.529	31.02	45.26
E_a (kJ mol^{-1}) ±SD	46.45±0.08	53.074±0.18	44.184±0.16
Ln A ± SD	10.00±0.03	19.52±0.06	21.84±0.06
$k_{5°C}$ 10^{-5} (h^{-1}) ± SD	0.37±0.02	0.53±0.07	0.34±0.05
$t_{90/5°C}$ (day) ± SD	1181±77	820±105	1278±183

organs and counteracting decomposition reactions by prolonging the stability of the liquid. The modification of the liquid using ions of zinc and selenium could reinforce the above effect. The current results indicate that the addition of zinc decreases the stability of the liquid by 30.5%, while the addition of selenium prolongs the stability by 8.21%.

Depending on the pH, ascorbic acid occurs in one of three different forms: H_2As (pH=0-3), HAs^- (pH=4-11) and As^{2-} (pH>11). In the tested liquids of pH=7.4, ascorbic acid is almost entirely dissociated (protons from the O-3 lactone ring are dissociated) and it occurs as HAs^- hydroanion. As a result of the reduction of two electrons in oxidation conditions, dehydroascorbic (DHA) acid is formed from HAs^-. This acid demonstrates the same biological activity as the reduced form. On the other hand, the hydrolysis of dehydroascorbic acid to 2,3-diketogulonic acid causes its degradation and depression of its antioxidant properties. It has been proven that zinc ions present in the solution increase the rate of the vitamin C decomposition reaction (Allwood et al. 1998).

The research conducted so far has proven that in solutions of pH > 5 including Se^{4+} ions and vitamin C, the reaction of the reduction to Se^0 does not occur (Ganther, Kraus 1989, Allwood, Kearney 1998, McGee et al. 1985). This is explained by the synergism of action of vitamin C and Se^{4+} antioxidant in the tested liquid.

Similar results have been obtained while examining the influence of Se^{4+} and Zn^{2+} on the stability of 0.3 mM vitamin C solution. It has been established that the addition of selenium increases the stability of vitamin C by 34%, while zinc decreases it by 23% (Dolińska et al. 2012*b*).

In living organisms, Na_2SeO_3 may undergo a two-stage reduction to hydrogen selenide H_2Se by means of glutathione reductase and NADPH. The product is then a selenium donor to the physiologically active selenocysteine occurring in the active site of selenoproteins. As a constituent of selenoproteins, selenium excites the immune system to increase the production of antibodies and enahnces activity in immune cells (Combs et al. 2001).

CONCLUSIONS

The results indicate that the addition of zinc decreases the stability of the liquid by 30.5%, while the addition of selenium prolongs the stability by 8.21%. This is explained by the synergism of action of vitamin C and Se^{4+} antioxidant in the tested liquid.

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