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PHYSICAL-CHEMICAL PROPERTIES AND THE REACTIVITY OF PYRIDOXINE AND PYRROLIDONE CARBOXYLATE AND THEIR PROTOLYTIC FORMS

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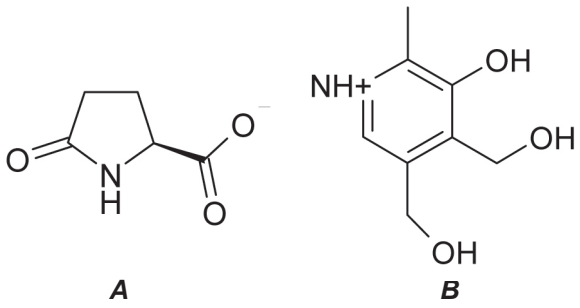
Preparation Methadoxine is equimolar salt, which cationic component (pyridoxine) is 3-oxypyridine derivative, possessing B_6-vitamine like activity, while anionic component is the cyclic lactame of glutamic acid. Since biopharmaceutical and pharmacological properties of this drug depend on biochemical transformation its components, of the aim of this work was to determine the structure of possible ionized pyridoxine and pyrrolidone carboxylate forms and their reaction ability in biochemical processes. Physical-chemical properties of compounds (pK_a, logP, logD, proton donor/acceptor quantity, solubility (g/l)) were calculated with ACD/pK_aDB program or obtained from Pub-Med physical/chemical properties database. UV spectra of compounds were obtained after dissolution in different pH solutions (1.0, 4.5 and 6.8). It was found that at different pH values one can observe changes of the absorption spectra due to the presence of prevailing amount of the protonated form. An analysis of both pK_a, logP and logD indicators and reactive functional groups of Methadoxine components has revealed that they can be protonated in different regions of gastro-intestinal tract, that influences their solubility in hydrophilic and lypophilic media. Pharmacological properties of pyridoxine and pyrrolidone carboxylate themselves are performed after their preliminary biotransformation to active metabolites. Only ionic interaction between Methadoxine components in the substance composition can appear, that provides its pharmaceutical stability and ensures its activity only in the organism conditions.

K e y w o r d s: pyridoxine, pyrrolidone carboxylate, protolytic forms of methadoxine, lipophilicity, biochemical activation.

A problem of therapy of hepatitis of different etiology is especially urgent. In spite of the great arsenal of hepatoprotectors clinicists cannot always achieve stabilization of hepatitis course, increase regenerative activity and prevent live fibrosis and cirrhosis [1-3]. In this connection a search for new medical agents with a broad range of pharmacological activity and economic accessibility continues. Pyridoxine-L-2-pyrrolidon-5-carboxylate (Methadoxine), which is a salt of pyrrolidone carbxylate (*A*) and pyridoxine (*B*), remains the object of researchers' attention for a long period of time [4-7].

It may be a priori suggested that such a combination has to be pharmacologically active. This is explained by the fact that L-2-pyrrolidon-5-carboxylate (pyroglutamate, 5-oxoproline), i.e. cyclic lactame of glutamic acid (intermediate link of gamma- glutamic cycle), which takes part in glutathione metabolism processes, is the molecule anion component [8]. A cationic component – a protonated form of pyridoxine – has vitamin activity (one of three forms of vitamim B_6). It plays the important role in metabolism, necessary for normal functioning of the central and peripheral nervous systems, participates in synthesis of neuromediators. Its phosphorylated form provides the processes of decarboxylation, reamination, desamination of aminoacids, takes part in synthesis of protein enzymes, hemoglobin, prostaglandins, in metabolism of serotonin, catecholamines, glutamic



A ***B***

acid, GAMK, histamine, improves metabolism of unsaturated fatty acids, decreases cholesterol and lipid level in blood [9, 10].

From the chemical point of view pyridoxine and pyrrolidone carboxylate in the drug composition are bound by salt formation (salification), and in this form their pharmacological properties are synergic. At the same time the ionic character of this compound foresees its dissociation in water medium and realization of pharmacological effect of separate preparation components.

In correspondence with the requirements of regulatory organs such a hybrid of compounds had been registered as a substance by the Laboratory Baldachi S p. A, Italy, and it was later used for making drugs in different countries. Methadoxine analogues Alcodez® IC and Liveria® IC, developed by Physical-Chemical Institute of NAS of Ukraine jointly with ALC "INTERCHEM", are produced in Ukraine.

Salification is one of the most common methods of increasing solubility, stability and keeping to stechiometry of medicines. At the same time, the equilibrium is established in the organism (blood plasma, intercellular space and cytosole of cells) between separate protolytic forms (protonated or deprotonated), which determines such processes as absorption (bioavailability), metabolism, distribution of substances among organs and tissues, ability to overcome biological barriers and interact with the corresponding targets. Moreover, both the components and their proteolytic forms can enter in chemical interaction that may reflect on quality of the ready-to-use medical form.

Allowing for all this, the work aim was to determine structures of possible ionized forms of pyridoxine and pyrrolidone carboxylate and their reactivity in biochemical processes, that is a necessary stage of substantiating expediency (efficiency and safety) of this medicine use.

Materials and Methods

The study deals with analysis of potential reactivity of pyridoxine and its participation in biochemical processes (its bioisosters – pyridoxal and pyridoxamine, as well as pyrrolidone carboxylate and its hydrolysis product – glutamic acid). Physical-chemical parameters of the compounds (ionization constant, pK_a lipophylicity, logP, lipophylicity at a certain pH, logD, the number of proton donors and acceptors, solubility (g/l) were calculated using the computer program ACD/pK_aDB and obtained from the database of physical-chemical properties of compounds PubMed. The choice of the above indices has been caused by their influence on the compound ability to dissolve in hydrophilic medium (the number of donors and acceptors, capacity to ionization at physiological pH (logD), to overcome biological barriers and to dissolve in nonpolar regions of lipid membranes (logP, logD, pK_a), as well as the drug ability to interact with the biological targets (molecule fixation in the active centre of receptor/enzyme owing to hydrogen links formed between donors and acceptors of protons). Due to calculation impossibility of lipophylicity values (logP) for ionized forms, this index was calculated only for the formal non-ionized structure, and logD values were obtained on the basis of Henderson-Hasselbalch equation.

UV-spectra of compounds were recorded after their previous dissolution in solutions with different pH values (0.1 M solution of hydrochloric acid (pH 1.0), 0.05 M acetate buffer (pH 4.5) and 0.05 M phosphate buffer (pH 6.8). An exact hinge of methadoxine (0.25 g) was dissolved in the corresponding solvent to concentration 0.1% and the absorption spectra on spectrophotometer Lambda-9 (Perkin-Elmer) (quartz cuvette, length 1.0 cm) were obtained

Results and Discussion

Pyridoxine is a stable precursor of active forms of vitamin B_6 – pyridoxale and pyridoxamine (Fig. 1). Since the use of each this form to achieve pharmacological effect is possible, the choice of a separate structure is based on its physical-chemical properties (Table 1).

Methadoxine components in water medium dissociate and form corresponding hydrated ions which can be at a certain distance from each other. Protonation/deprotonation of pyridoxine and pyrrolidone carboxylate considerably affects physical-chemical indices of compounds and changes inversely their structure that is reflected on their absorption spectra in UV-region. Since the extent of absorption from different sections of gastrointestinal tract (GIT) depends on the balance of ionized and unionized forms, characteristic of physical-chemical properties of the studied compound is necessary. For this purpose the absorption spectra of methadoxine substance have been analyzed in solutions with different pH values, that correspond to the lowest value of stomach content (pH 1.0) and distal regions of GIT (5.5 of acetate buffer and 6.8 of phosphate buffer).

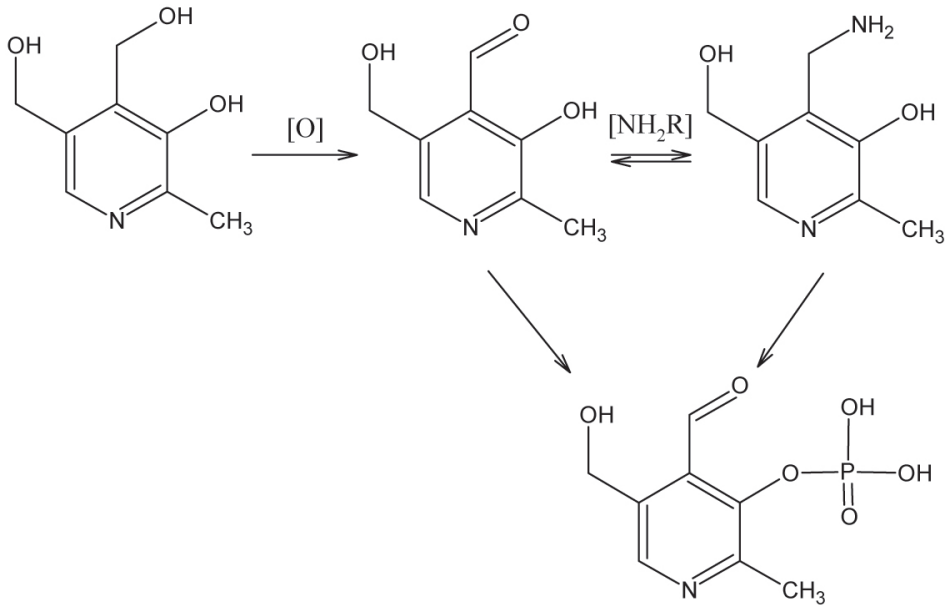
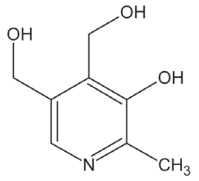
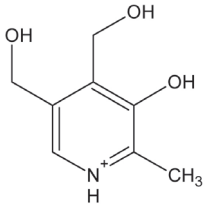
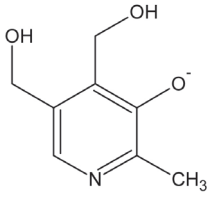


Fig. 1. Diagram of interrelations of different forms of pyridoxine in organism

Table 1. Structure, physical-chemical properties of pyridoxine and its protolytic forms

Form	Ionization constant, pK_a	Lipophilicity	Lipophilicity at a certain level of pH, logD	Quantity of proton donors	Quantity of proton acceptors	Solubility, g/l, (calcul.)
	–	-1.10 ± 0.25	–	3	4	220 (experim.)
	5.06 ± 0.28	–	-1.5	4	3	144.96
	8.37 ± 0.20	–	-1.53	2	4	154.74

Thus in acid medium (solution 0.1 M HCl, pH 1, Fig. 2) one can observe only one peak of absorption in the range of 290 nm, that may correspond to n→π* transition of electrons of carbonyl (C=O) of pyrrolidoncarbonic acid, which is in non-ionized state (pK_a = 3.47 ± 0.20). As is seen (Fig. 2), the increase of medium pH (acetate buffer pH 4.5) leads to the shift of this absorption band in low-enerhy region (bathochromic shift) and decrease of its intensity at the expense of the change of relation of concentrations of different protonated forms. At a higher pH (phosphate buffer, pH 6.8) deprotonation of heteroatom of aromatic system (gypsochrome shift to 240 nm) and presence of anion of pyrrolidoncarbonic acid takes place (the absorption band at 330 nm, Fig. 2)

Each of biochemically equivalent forms of vitamin B_6 may be potentially used in methadoxine composition. However, ionization (protonation by heteroring nitrogen) of pyridoxine is performed at a higher pH (pK_a = 5.06 ± 0.28), than other compounds (pyridoxale and pyridoxamine have values pK_a 3.26 ± 0.28 and 2.45 ± 0.46, respectively), as a result most part of the compound exists in ionized form, that improves considerably its solubility in water medium (Fig. 3). Another monoprotonated form (pK_a 6.54 ± 0.50) also exists for pyridoxamine, as a result it exists at physiologic pH in considerable quantity as a charged part, that can limit its ability to cross biological barriers.

Of three noted bioisosters the logD value exceeds 0 for pyridoxine (i.e., in the limits of pH from ~4.4 to ~8 its concentration in hydrophylic and lipophylic phases is comparable). Availability of proton donors and acceptors for pyridoxine is the highest at a higher pH (among above compounds the highest value of pK_a corresponds to 5.06 ± 0.28), that is why the equilibrium between protolytic forms for it is established more quickly in conditions of physiological pH. It should be noted that the availability

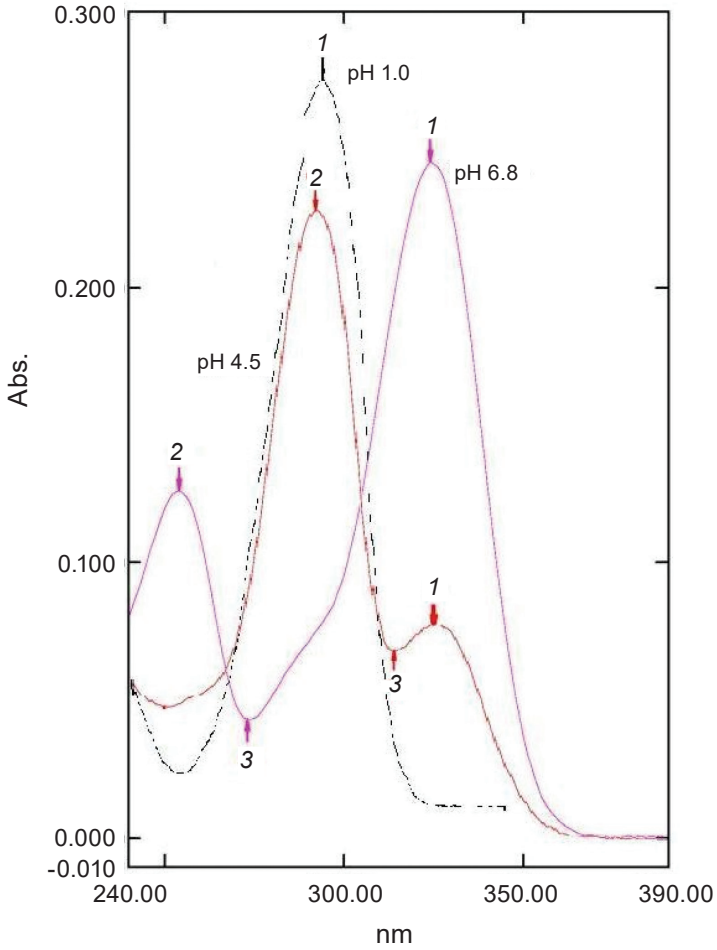
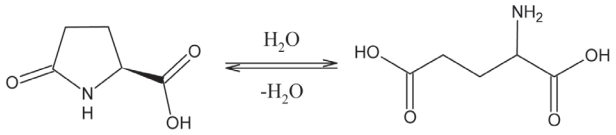


Fig. 2. Methodoxine absorption spectra in water solutions with different pH value: 0.1 M HCl pH 1.0; 0.05 M acetate buffer – pH 4.5; 0.05 M phosphate buffer – pH 6.8

of a higher quantity of potential proton donors in these pH limits is a considerable solubility factor in biological liquids, than quantity of proton acceptors. Besides, aldehide group of pyridoxale is more able to oxidation (with formation of carboxylate), which does not possess vitamin activity, thus, proceeding from the above said the use of pyridoxine is more expedient from the viewpoint of pharmacology.

Methadoxine anion component is presented by pyrrolidone–carboxylate, or pyrrolidone-carboxylic acid which persists in organism in normal physiological conditions. Pyrrolidone-carboxylic acid precursor is glutamic acid, which is not only a component of numerous proteins, but manifests itself various physiological effects. If glutamic acid is present in N-terminal end of peptide chain, it creates pyrrolidone-carboxylic acid, which availability, in particular, plays an important part in protein synthesis [11]. Synthesis of glutamic acid from pyroglutamic acid is a spontaneous (chemical) process, however the reaction in the organism is power-dependent and is catalyzed by 5-oxoprolinase.

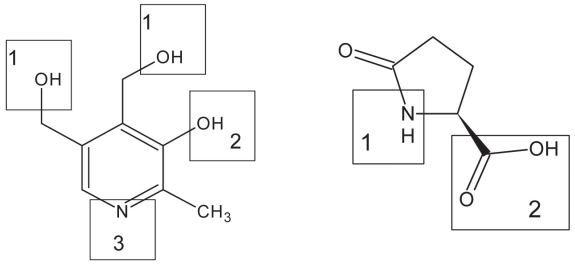


Inverse reaction (cyclization of glutamic acid with formation of lactam) is catalyzed by glutamyl cyclase [11, 13].

In contrast to glutamic acid only ionization of carboxyl group in pyrrolidone-carboxylic acid is a significant parameter in physiological conditions (pK_a = 3.47 ± 0.20), while protonation and deprotonation of amide atom of nitrogen can be realized at very low or very high pH values (-1.88 ± 0.40 or 12.76, respectively). This means its presence in the organism mainly in the form of anion. In contrast to pyrrolidon-carboxylic acid glutamic acid is intensively used in biochemical processes (utilization of ammonium, reamination, ketoglutarate pool replenishment, etc.); that is why just the use of pyroglutamic acid is more expedient, since this gives a possibility to sustain its concentration in blood at a higher level (Fig. 3) and its further supply to the brain for implementing neuroprotecting action.

Data presented characterize methadoxine as a dynamic system of pyridoxine and pyroglutamate, the acid-base interaction being possible in it. Except for the above types of interaction, the components are capable of the other ones that determines a necessity to characterize the reaction ability of chemical groups and conditions of the course of possible reactions.

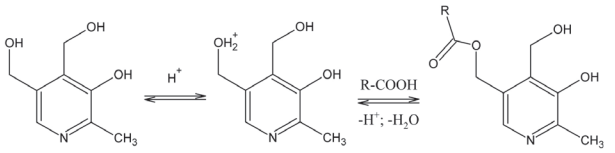
In pyridoxine molecules (*A*) one can distinguish three kinds of reaction groups: 1 – alcohol aliphatic, 2 – phenol hydroxyl, 3 – nitrogen of aromatic system, while in the molecule of pyrrolidone-carboxylic acid (*B*) that is only amide group (1) and carboxylic one (2), which determine potentiality of the following reactions:



A *B*

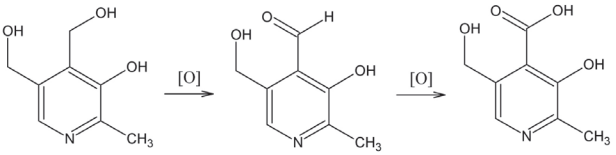
Reactions by aliphatic hydroxyl:

Formation of esters with carboxylate-anion is possible in the reactions of nucleophylic substitution (which are preceded by hydroxyl protonation):



Taking into account high concentration of pyrrolidone carboxylate in methadoxine substance, this reaction is more expected in the organism and under long-term storage of the pharmaceutical preparation. Since esters, which are formed, are labile compounds, in the organism conditions (both at the stage of absorption and distribution) one should expect a leftward shift of the reaction equilibrium and hydrolysis of the formed ester.

Oxidation of aliphatic group:



Owing to chemical stability of aliphatic hydroxygroup in conditions of storage of the ready-to-use medical form, this reaction takes no place, but in conditions *in vivo* it determines pharmacological effect of pyridoxine, which vitamin activity is reali-

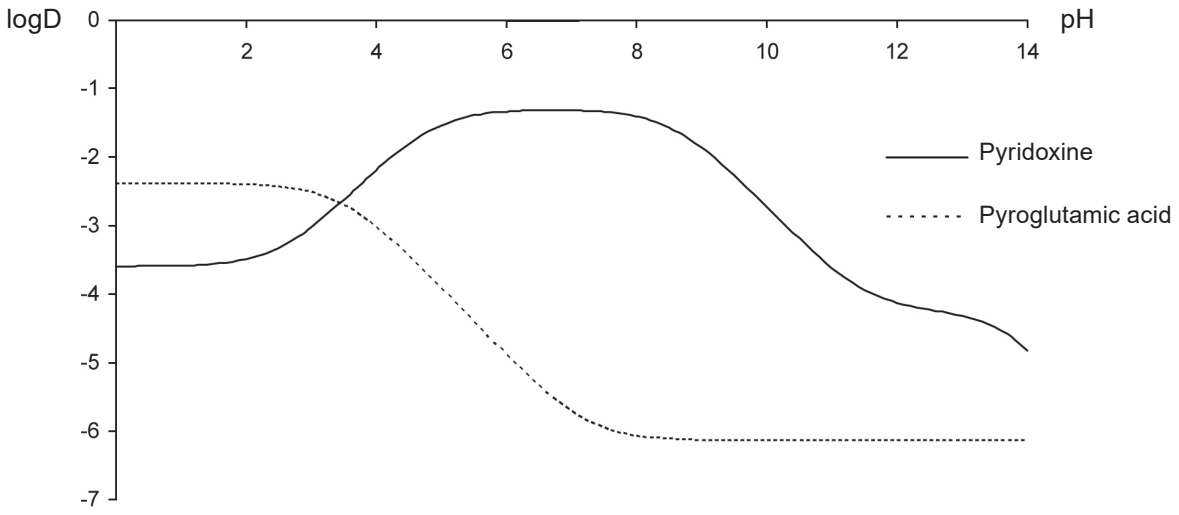
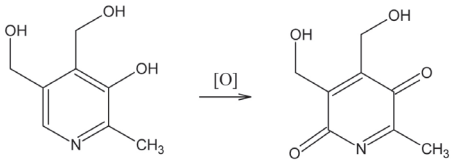


Fig. 3. Change of logD value for pyridoxine and pyroglutamic acid depending on pH value

zed due to oxidation to aldehyde form, which enters in the reactions of reamination, decarboxylation, etc.

Oxidation by phenol hydroxyl:

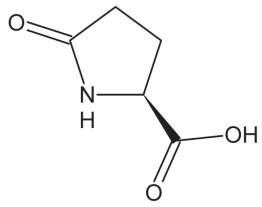
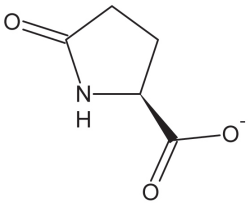
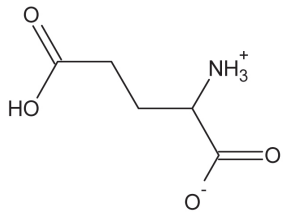
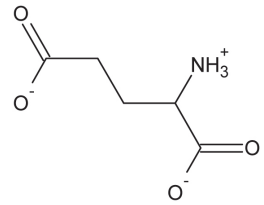


Phenol hydroxyl is a rather stable group in the acid medium and it is sensitive to oxidative agents in the basic (increase of phenolate-anion concentration) one. In conditions of the substance storage the ionization by phenol hydroxyl is considerably decreased due to availability of a more acid proton of carboxylic group of pyrrolidone-carboxylic acid (pK_a 8.37±0.2 and 3.47±0.2, Tables 1, 2, respectively). In the organism conditions pyridoxine oxidation to corresponding quinone may be one of this vitamin inactivation ways.

Hydrolysis reactions may take place only for pyrrolidone carboxylic acid, cyclic amide group being present in its molecule. However, as was noted, fermentative formation of glutamic acid is also one of the processes, through which pharmacological action of methadoxine is realized.

We have no technical means to confirm: which of the above protolytic forms of pyridoxine and pyrrolidone carboxylate correct to a higher extent the particular metabolic processes in conditions of pathology. But on the basis of physiologic response, obtained in experiments on animals and patients under methodoxine administration, one can notice that chemical and biochemical transformation is a necessary stage of development of the pharmacological spectrum of action. 1. Antioxidant action. Oxidative stress in people is a cause or important component of serious diseases. From the viewpoint of chemistry the oxidative stress is an essential increase of the cell redox-potential or essential decrease of restoring ability of redox-pairs, such as oxidized/restored glutathione. Methadoxine stabilizes activity of glutathione-reductase – the enzyme, which renews disulphide bonding of oxidized glutathione GSSG to its sulfhydryl form GSH. [14]. Methadoxine molecule in tissues may metabolize chemically, forming corresponding N-oxide, which function structurally as a spin trap, for reactive oxygen forms. 2. Antisteatosis action. Methadoxine can inhibit accumulation of monounsaturated and saturated fatty acids in cells of the brain, heart, liver [15, 16]. This action decreases negative effect of alcohol and other toxic factors on the organism [16]. 3. Influence on energetic balance. Alcohol intoxication leads to the decrease of ATP production in the brain and liver. The renewal of ATP amount under the drug administration was established in experiments on animals [17]. 4. Antifibrotic action. Some experiments have shown [4], that methadoxine blocks collagen synthesis in Ito cells. This effect was studied in detail on the models of liver fibrosis with the use of toxic carbon tetrachloride, as well as under the use of ligature of the bile duct. 5. Effect of desintoxication. The drug affects alcohol dehydrogenase activity. The enzyme level in the organism decreases under chronic alcohol intoxication. Under drug taking its level considerably increases and achieves normal indices [18].

Table 2. Physical-chemical properties of pyroglutamic and glutamic acids and their protolytic forms

Form	pK_a	logP	logD	Quantity of proton donors	Quantity of proton acceptors	Solubility, g/l, (calcul.)
Pyroglutamic acid						
	–	2.39 ± 0.27 (theor.) -0.86 (exp.)	–	2	4	476 (exp.)
	3.47 ± 0.20 (3.48)	–	-2.7	1	4	987 (theor.)
Glutamic acid						
	2.17 ± 0.10	–	-4.13	2	3	96.87
	4.57 ± 0.10	–	-4.21	1	4	103.43

The above properties of methadoxine components and their protolytic forms determine pharmacokinetic parameters of the drug: bioaccessibility (60-80%), half-life period 40-60 min, binding with plasma proteins 50% [19]. Thus, equimolar combination of pyrrolidone carboxylate and pyridoxine in the composition of methadoxine has necessary pharmacodynamic and pharmacokinetic properties, which expand its use in medical practice. Besides the liver alcohol disease and alcohol dependence the drug is also used at non-alcohol pathology, at virus hepatitis C, for side-effects reducing of anti-viral, oncologic and centrally-acting neurotropic drug administrations.

Thus, pyrrolidone carboxylate and pyroglutamate, which are active components of the medical drug – methadoxine in different regions of GID are subject to protonization, which affects their solubility in water and lipid media, ability to overcome biologic membranes and manifest properties of proton donors or acceptors. Pyrridoxin and pyrrolidone carboxylate own pharmacologic effects in the organism conditions are realized after their preliminary biotransformation to physiologically active metabo-

lites. Interaction between methadoxine components in the substance composition is possible only at the level of ions that provides pharmaceutical stability of the substance and display of activity only in the organism conditions.

ФІЗИКО-ХІМІЧНІ ВЛАСТИВОСТІ ТА РЕАКЦІЙНА ЗДАТНІСТЬ ПІРИДОКСИНУ І ПІРОЛІДОНУ КАРБОКСИЛАТУ ТА ЇХ ПРОТОЛІТИЧНИХ ФОРМ

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Препарат Метадоксин – еквімолярна сіль, катіонний компонент якої (піридоксин) є похідним 3-оксипіридину, що виявляє вітамінну активність (B_6), у той час як аніонний компонент – це циклічний лактам глутамінової кислоти. Оскільки біофармацевтичні та фармакологічні характеристики цього препарату залежать від біохімічної трансформації його компонентів, метою роботи було визначення структури можливих іонізованих форм піридоксину і піролідону карбоксилату та їх реакційної здатності в біохімічних процесах. За допомогою комп'ютерної програми ACD/pK_aDB та бази даних фізико-хімічних властивостей сполук PubMed розраховано фізико-хімічні параметри сполук (pK_a, logP, logD, кількість донорів і акцепторів протонів та розчинність (г/л)). Одержано УФ-спектри досліджуваних сполук, розчинених у буферних розчинах із різним значенням pH (1,0; 4,5 та 6,8). Встановлено, що за різних значень pH спостерігаються зміни спектрів поглинання, які пов'язані з присутністю переважної кількості протонованої форми. Аналізом показників pK_a, logP та logD, а також реакційноздатних функціональних груп компонентів Метадоксину визначено, що вони піддаються протонізації в різних відділах ШКТ, яка впливає на їх розчинність у водному та ліпідному середовищах. Власні фармакологічні ефекти піридоксину та піролідону карбоксилату в умовах організму реалізують після їх попередньої біотрансформації до фізіологічно активних метаболітів. Взаємодія між компонентами метадоксину в складі субстанції відбувається на іонному рівні, що забезпечує фармацевтичну стабільність субстанції та її активність лише в умовах організму.

К л ю ч о в і с л о в а: піридоксин, піролідону карбоксилат, протеолітичні форми метадоксину, ліпофільність, біохімічна активація.

ФИЗИКО-ХИМИЧЕСКИЕ СВОЙСТВА И РЕАКЦИОННАЯ СПОСОБНОСТЬ ПИРИДОКСИНА И ПИРРОЛИДОНА КАРБОКСИЛАТА И ИХ ПРОТОЛИТИЧЕСКИХ ФОРМ

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Препарат Метадоксин – эквимолярная соль, катионный компонент которой (пиридоксин) – производное 3-оксипиридина, проявляющее витаминную активность (B_6), тогда как анионный компонент – это циклический лактам глутаминовой кислоты. Поскольку биофармацевтические и фармакологические характеристики этого препарата зависят от биохимической трансформации его компонентов, целью работы было определение структуры возможных ионизированных форм пиридоксина и пирролидона карбоксилата и их реакционной способности в биохимических процессах. С помощью компьютерной программы ACD/pK_aDB и полученных из базы данных физико-химических свойств соединений PubMed были рассчитаны физико-химические параметры соединений (pK_a, logP, logD, количество доноров/акцепторов протонов и растворимость (г/л)). Получены УФ-спектры исследуемых соединений, растворенных в буферных растворах с различным значением pH (1,0; 4,5 и 6,8). Установлено, что в указанном интервале pH наблюдаются изменения спектров поглощения, связанные с присутствием наибольшего количества протонированной при данном pH формы. Анализом показателей pK_a, logP и logD, а также реакционноспособных функциональных групп компонентов Метадоксина определено, что они подвергаются протонированию в различных отделах ЖКТ, что влияет на их растворимость в гидрофильной и липофильной средах. Собствен-

ные фармакологические эффекты пиридоксина и пирролидона карбоксилата в условиях организма реализуются после их предварительной биотрансформации в физиологически активные метаболиты. Взаимодействие между компонентами метадоксина в составе субстанции происходит на ионном уровне, что обеспечивает фармацевтическую стабильность субстанции и проявление активности только в условиях организма.

Ключевые слова: пиридоксин, пирролидона карбоксилат, протеолитические формы метадоксина, липофильность, биохимическая активация.

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