Influence of nature and macromolecular characteristics of carrier polymer on immobilization of bolaform ions

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Abstract. The study of the interactions of polyelectrolytes with organic ions and especially with ions of physiologically active substances is of particular. There are wide ranges of polymers used for these purposes, the most convenient "substrate" is considered to be synthetic carriers that are easily regulated during production. The object of research was the antiprotozoal drug azidine, widely used in veterinary practice, which, when dissolved in water, forms a complex bolaform cation: 4.41-diamidinodiazoaminobenzene. To develop a prolonged dosage form for veterinary medicine, the binding of this substance with various synthetic polymers such as polyvinylpyrrolidone, polyvinyl alcohol, polyacrylic, and polymethacrylic acids are important. In addition; their copolymers, was studied using physical and chemical methods. The influence of the nature of the carrier polymer, the molecular weight, and the conformation of macromolecules on the process of binding organic ions were also examined.The results obtained were confirmed by in vivo tests on cattle. A pattern is shown between the strength of the bond of the drug substance with the polymer carrier and the time of its preventive action.

1 Introduction

Protozoal diseases are widespread animal diseases. The damage these diseases cause to livestock is enormous, which undoubtedly justifies the search for highly effective chemotherapy drugs to treat animals with these diseases.

In veterinary medicine, antiprotozoal drugs are widely used to treat cattle: azidine, which is used respectively in the form of 7 and 5% aqueous solutions by subcutaneous injection for babesiosis, francaillosis, and trypanosomiasis in cattle. According to preliminary data, their mechanism of action is based on the penetration of medicinal substances (DS) into piroplasmids. It has a pronounced chemotherapeutic effect, but due to

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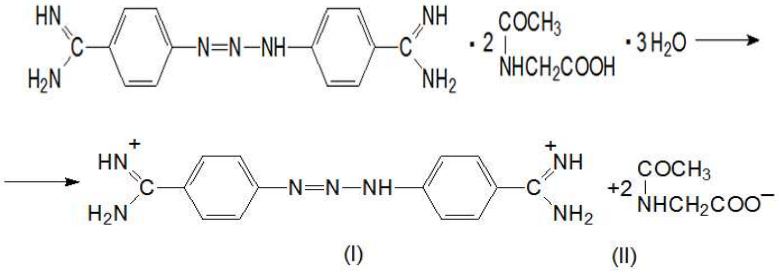
the relatively rapid decrease in its concentration in the bodies of animals, it must be administered every 10–12 or 7-8 days, respectively, which creates certain difficulties, especially in grazing livestock [1].

The last decades have seen the rapid development of one of the branches of chemical science, polymer chemistry, which is associated with its use in various industries and human activities. For example, polymers have found wide application in medicine and pharmacology, in particular in veterinary practice. A large percentage of packaging materials used in medicine are polymers. Polymers are also widely used in the manufacture of auxiliary materials and medical products. Polymers are widely used in the development and use of drugs with unique properties (low toxicity, prolonged action, manifestation of new physiological properties, etc.), which are a consequence of the macromolecular nature of the drug carrier [2]. However, there are still many aspects that require in-depth study when choosing polymers as carriers of physiologically active compounds, some of which are the subject of these studies.

2 Methods

The work used such physicochemical methods as equilibrium dialysis, potentiometry, differential calorimetry, viscometry, and velocity sedimentation. The preventive effectiveness of polymer dosage forms of azidine was studied on black and white bulls at the age of 12 months. During the research, modern methodologies used in polymer chemistry and pharmacology were used to detect patterns in the studied drug and polymer systems. From a chemical point of view, azidine is a complex salt of 4.41-diamidinodiazoaminobenzene with aceturic acid. The active ingredient is 4.41-diamidinodiazoaminobenzene (hereinafter referred to as azidine), a yellow powder that is poorly soluble in water and organic solvents and stable in a neutral environment [3]. In water, azidine dissociates to form a complex bolaform cation (I) and an aceturic acid anion (II).

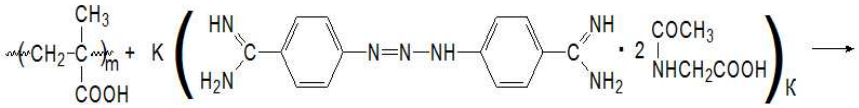


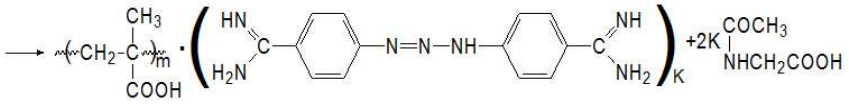
(I) (II)

According to its chemical structure, azidine belongs to one class of organic bases: diamidines.

A very intense binding of azidine by polymer carriers is observed in the structure of macromolecules of ionizable groups capable of strong ion-ion interactions with oppositely charged groups of the drug.

In general, the range of types of interactions between a polymer and an organic substance is quite wide, but the contribution of each may be different. The interaction of PMAA with azidine can be represented by the following scheme:





3 Results and Discussion

In order to create prolonged forms of azidine, taking into account the chemical structure and mechanism of its action, the interaction of the latter with the following water-soluble polymers was studied: polyvinylpyrrolidone (PVP), polyvinyl alcohol (PVA), polyacrylic (PAA), and polymethacrylic acids (PMAA), as well as some of them copolymers. As is known, the correct choice of carrier polymer when creating polymer dosage forms is of decisive importance. Although the PVP molecule does not have functional groups capable of participating in ion-ion interactions, the binding of the drug occurs quite intensively (Figure 1). Thus, the change in enthalpy (ΔH) upon binding of azidine to PVP, determined by differential calorimetry, turned out to be equal to 1.9 kcal/mol, which indicates predominantly hydrophobic retention of organic molecules on the polymer matrix [2]. The binding ability of PVA can be explained by the presence of hydroxyl groups in it, which, when interacting with azidine molecules, can form additional hydrogen bonds [3-4], as evidenced by the ΔH value of the process, which is equal to 3.2 kcal/mol.

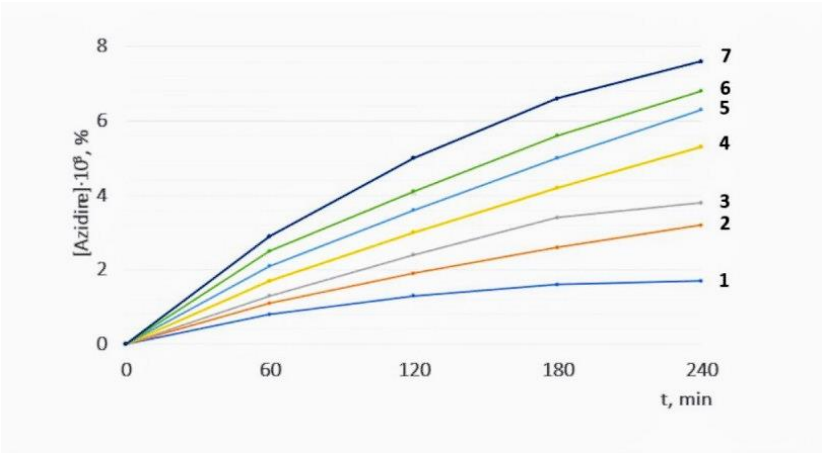


Fig. 1. Kinetics of the dialysis of azidine into aqueous solutions: 1: into water; 2: PVP; 3: PVA; 4: copolymer of acrylic acid with acrylamide; 5: PAK; 6: PMAC; 7: copolymer of methacrylic acid with maleic acid. The concentration of polymers is 1.16·10-3 mol/l, and the concentration of azidine in the original solution is 1.77·10-3 mol/l. T = 303 K.

As a result of the interaction of the components, an equivalent amount of free aceturic acid is formed, which leads to a decrease in the pH of the solution. The interaction between PMAA and azidine is confirmed by potentiometric titration data, which makes it possible to determine the amount of bound azidine (Figure 2).

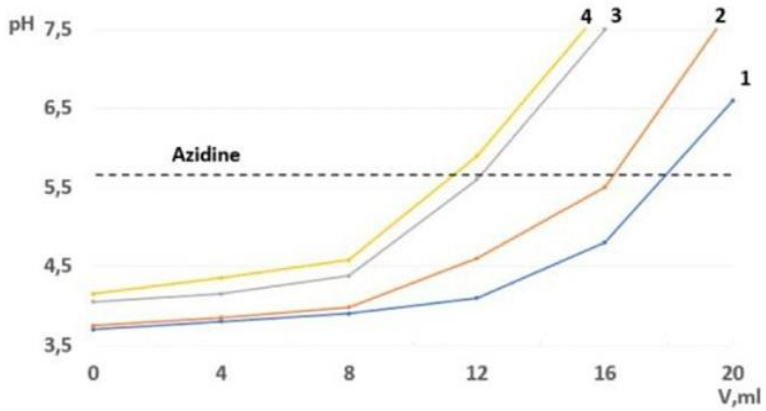


Fig. 2. Potentiometric titration curves of solutions after complexation of azidine with the polymer: 1: PAA; 2: AK-AA copolymer; 3: PMAK; 4: MAK-MK copolymer, solution 0.013 N. NaOH. The dotted line is the pH of an aqueous solution of azidine. The drug/polymer ratio is 0.384 mol/basic mol. T = 298 K.

The largest amount of aceturic acid is released when azidine reacts with PAA; interaction with PMAA leads to a smaller decrease in pH. The difference in the reactivity of PAA and PMAA is due to intramolecular interactions and the high acidity of PAA. It is known that the average radius of gyration of PAA macromolecules is slightly higher than that of PMAA due to hydrophobic interactions with α-methyl groups in the macromolecules of the latter.

The denser coil of the PMAA macromolecule compared to PAA obviously represents a greater obstacle to the bound azidine molecules. The lower binding capacity found for the copolymer of acrylic acid with 44 mol% acrylamide (AA-AA) than PAA is due to a decrease in the number of ionizable sites in the macrochain. The decrease in the binding capacity of the polymer upon transition from PMAA to a copolymer of methacrylic acid with 13 mol.% maleic acid (MAA-MA) is apparently explained by the insufficient involvement of the functional groups of the second comonomer in the interaction with the studied bolaform ion [4].

The study of the kinetics of azidine binding on these polymers by the method of stationary sorption confirmed the correct arrangement of the polymers according to their binding ability: PAA > AK-AA copolymer > PMAA > MAA-MK copolymer (Figure 3).

Consequently, the effective binding of low molecular weight substances is determined not only by the number of functional groups in the carrier polymer, which must be necessary and sufficient, but also by the conformational state of macromolecules, determined by the presence of other groups of atoms. In this regard, synthetic polymers, unlike natural ones, have great advantages, since through synthesis it is possible to change the chemical composition, macrochain length, physicochemical and biological properties of carrier polymers [5].

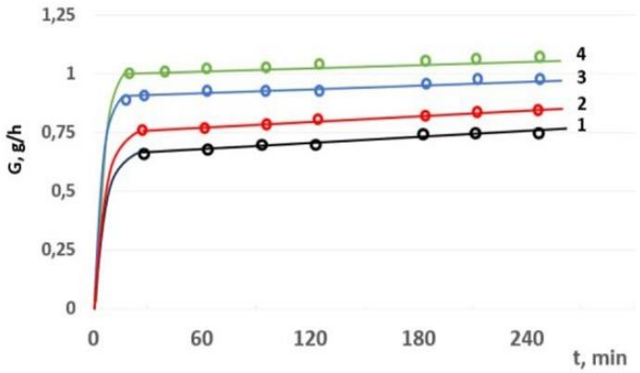


Fig. 3. Kinetics of binding of azidine* from aqueous solutions to polymers: 1 - MAK-MK copolymer; 2 - PMAK; 3 - copolymer AK-AA; 4 - PAK. The polymer concentration is 0.029 basic mol/l, the concentration of the starting azidine is 8.9·10-3 mol/l. T = 298 K.

An interesting pattern in the dependence of the binding ability of macromolecules of different molecular weights was discovered in [6]. To study the influence of this parameter of PMAA on its complex-forming ability, we used polymer fractions isolated by fractional precipitation from a polymer obtained by radical polymerization [7]. In Figure 4 shows the dependence of the thermal effect of the interaction of the polymer with azidine on the molecular weight of PMAA. It can be seen that with a decrease in molecular weight, the reactivity of macromolecules increases slightly, apparently due to the greater "looseness" of the coils of macromolecules. It is interesting to compare the values of the thermal effects of complexation of syndiotactic and atactic polymers. The observed difference is likely caused by a decrease in steric hindrance upon interaction of the syndiotactic polymer with organic ions. A similar picture of the difference in the reactivity of a syndiotactic polymer versus an atactic one with respect to bolaform cations was recorded in [8].

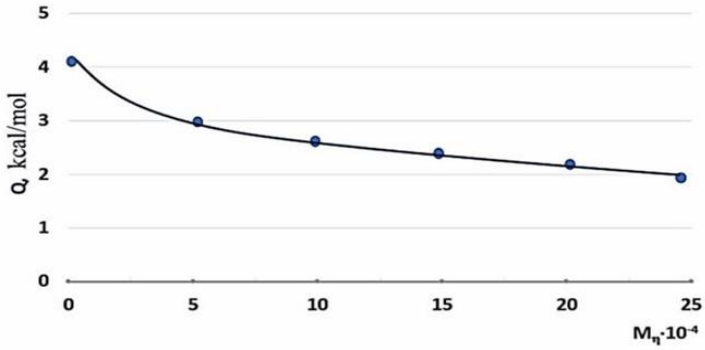


Fig. 4. Dependence of the thermal effect of the interaction between aqueous solutions of PMAA and azidine on the molecular weight of the polymer. 1 - for unfractionated atactic PMAC; 2 - for unfractionated syndiotactic PMAC. The azidine/PMAA ratio is 0.0077 mol/basic mol. T = 308 K.Equations and mathematics.

The effect of the molecular weight of the polymer on its binding ability was confirmed by viscometric study (Figure 5). A decrease in the relative change in the specific viscosity of the solution when adding the same amount of azidine to PMAA fractions with different molecular weights made it possible to record a stronger change in the hydrodynamic volume of macromolecules with lower molecular weight. This interpretation of the observed results finds an analogy in the literature [4].

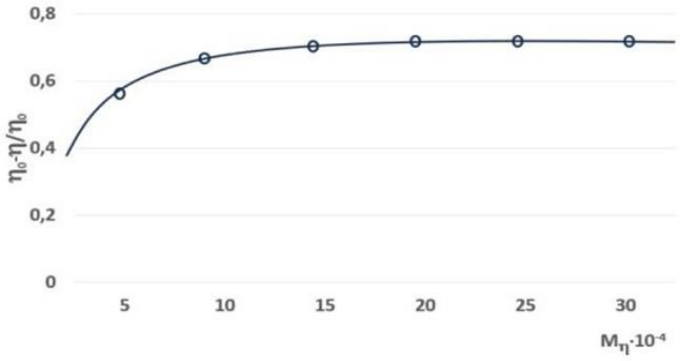


Fig. 5. Dependence of the relative change in the specific viscosity of a solution of the polymer complex of azidine with PMAA on the molecular weight. 1 - polymer fractions; 2 - unfractionated polymer; 3 - syndiotactic polymer. The azidine/polymer ratio is 0.0127 mol/basic mol. T = 298 K.

Certain information about the intensity of interaction of macromolecules with low molecular weight organic ions can be obtained by the method of velocity sedimentation. But, at the same time, its value, as can be seen from the formula below, is directly proportional to the hydrodynamic characteristics of macromolecules [9]:

- $S \approx V_S/w^2 \cdot r \approx (1-\tilde{v}\rho)M/N_A \cdot f$.
- $\tilde{v}$ - specific partial volume; ρ is the density of the solution.
- M is the molecular weight of the polymer; NA - Avogadro's number.
- f is the friction coefficient.

Thus, when diamidines interact with functional polymers, they are immobilized on macromolecules. At certain drug/PMAA ratios, precipitation of the polymer complex is observed in both systems. Subsequent sorption of the drug on the precipitated polymer complex occurs much more slowly. At the stage of formation of sufficiently hydrophilic soluble complexes (at a low value of drug/PMAA), strong binding of drug molecules to the macromolecule occurs, simultaneous compaction of its coil.

The latter, obviously, should be reflected in some properties, in particular sedimentation ones. The sedimentation coefficient of the polymer complex increases with increasing drug concentration in the solution; in addition, a certain pattern can be traced in the magnitude of the relative change in the sedimentation and sorption coefficient from the molecular weight of the polymer (Table 1, Figure 6). It can be seen that the relative change in the sedimentation coefficient of low molecular weight fractions during complex formation is more significant.

Table 1. Dependence of changes in the sedimentation coefficient of the azidine-PMAA polymer complex on the molecular weight of the polymer. (azidine/PMAA ratio 0.05 mol/bas-mol).

$\bar{M}\eta$	$S_{pol.}$, sv	S_{komp}, sv	$S_{pol.}$- $S_{komp.}$/ $S_{pol.}$
16600	24.76	28.36	-0.1454
72750	27.50	29.28	-0.0674
121500	38.32	39.60	-0.0334
235000	59.70	61.28	-0.0264

The increase in the binding capacity of PMAA azidine during the transition from atactic to syndiotactic polymer was also confirmed by the steady-state sorption method [14-16] (Figure 6).

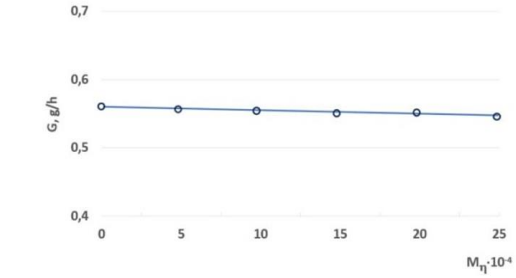


Fig. 6. Dependence of azidine binding on PMAA on the molecular weight of the polymer. 1 - unfractionated PMAC; 2 - syndiotactic polymer. The azidine/polymer ratio is 0.36 mol/basic mol. T = 298 K.

The preventive effectiveness of polymer dosage forms of azidine was studied on black-and-white bulls at the age of 12 months, previously tested for the absence of piroplasmids. Experimental bulls were injected with a suspension of the polymer complex into the upper third of the neck subcutaneously at a dose of 5 mg/kg (PMAC - azidine) and 4 mg/kg (PMAC - imidocarb). After 7, 14, 21, 28, 35, 40 days, the animals were infected subcutaneously with invasive blood in a dose of 20 ml taken from a patient with piroplasmosis and animal francaiellosis (Table 2).

Table 2. Chemopreventive efficacy of polymer complexes of azidine and imidocarb against mixed invasion (pyroplasmosis and francaiellosis) of cattle.

A drug	Contents in the preparation		Action time	We enter the quantity. Preparation, ml/100kg
	Polymer mg/ml	LV, mg/ml		
Control (azidine)	-	35	5-6	7.14
PVP+azidine	300	35	7-8	7.14
PVS+azidine	70	33	7-8	7.57
Poliglyukin+azidine	50	36	14-15	7.0
PMAK+azidine	80	60	24-28	4.15

Observations were carried out daily: a clinical examination, measuring body temperature, taking blood smears and viewing it under a microscope to detect the presence of piroplasmids. The duration of observation was 20 days [10]. For comparison, azidine polymer complexes based on PVP, PVA and polyglucin were also tested (Table 2). As a result of the studies, it was possible to discover a pattern between the strength of the bond between the drug and the polymer carrier and the time of its preventive action (Table 2). The strength of the drug-polymer bond was judged from calorimetric studies. Pharmacological studies in vivo on various animals confirmed the feasibility of choosing PMAC as an azidine prolongator, since the toxicity of polymer dosage forms turned out to be much less than that of the original drug [11-16] .

Thus, the conducted studies give reason to assume that the binding of azidine to PMAA is reversible and can be used to solve a specific problem - prolongation of its physiological action in order to provide effective treatment and prevention of protozoal diseases in cattle [16-18].

4 Conclusions

Based on the conducted research, the following conclusions can be drawn: - the presence of active functional groups in macromolecules greatly affects the binding ability of polymers

of organic molecules, an increase in the molecular weight of macromolecules slightly reduces the immobilizing ability of polymers, using PMAA as an example, a slight increase in the binding capacity of the polymer during the transition from atactic to syndiotactic is shown. for maximum binding of organic ions in macromolecules, the presence of functional groups in sufficient and necessary quantities is required,the binding of organic ions in the systems under study occurs “softly” due to ion-ion and hydrogen bonds, as well as hydrophobic-hydrophilic interactions. Thus, the conducted studies give reason to assume that the binding of azidine to PMAA is reversible and can be used to solve a specific problem-the prolongation of its physiological action-in order to provide effective treatment and prevention of protozoal diseases in cattle.

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