Complex compounds of mesalazine with cyclodextrins and the prospect of their application in the ulcer disease treatment

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Abstract. Mesalazine is highly effective in the treatment of ulcerative colitis and Crohn's disease. However, in view of the fact that this compound is easily absorbed in the upper small intestine and cannot reach the large intestine at a therapeutic concentration, it is relevant to enclose it in the cavity of cyclodextrins through the formation of inclusion complexes of the "guest–host" type. Mesalazine complexation with α-, β-, γ- cyclodextrins was studied by spectrophotometric method in aqueous solutions. We established that the composition of the resulting complexes is 1:1. In the temperature range 296 - 321 K, the stability constants are calculated, and the thermodynamic parameters of complex formation are determined. We developed a technique for the synthesis of complexes, and a prototype, and studied its antiulcer activity. The authors established that the complex of mesalazine with β- cyclodextrin has a more pronounced antiulcer activity compared to the original substances, and approximately the same action as the reference drug (Omez). However, the amount of mesalazine in the complex was only 12% wt., the remaining 88% wt. account for β-cyclodextrin. The results obtained indicate the possibility of complex formation between mesalazine and α-, β-, γ- cyclodextrins. The resulting complex compounds can become the basis of new anti-inflammatory and antiulcer drugs.

1 Introduction

Currently, mesalazine (MS) - 5-aminosalicylic acid - is the main component of drugs used in the treatment of inflammatory bowel diseases such as ulcerative colitis and Crohn's disease. Numerous studies have proven the effectiveness of MS oral administration in both induction and maintenance of remission in patients with mild to moderate activity colitis [1–4]. According to the scientists [5-8], mesalazine exhibits a local anti-inflammatory effect, so the concentration of the drug in contact with the damaged intestinal wall mucosa and the duration of this contact are extremely important. Since this compound is predominantly absorbed in the upper parts of the small intestine, its concentration in the affected areas of the colon is significantly lower than the therapeutic one [5-7]. The solution to the problem of premature

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absorption of 5-aminosalicylic acid can be the preparation of its inclusion complexes of the "guest–host" type with molecules of natural cyclic oligosaccharides, cyclodextrins (CD).

The first three representatives of the homologous series of cyclodextrins are the most studied and in demand. They are formed by six, seven and eight D - glucopyranose units linked by an α-1,4-glycosidic bond and are named, respectively, α-, β- and γ- cyclodextrins (α-, β- and γ- CD) [9, 10]. Due to their unique structure, CDs are capable of forming hydrophilic inclusion complexes with various organic and inorganic compounds, including pharmacologically significant ones. Complexation with cyclodextrins improves the solubility of medicinal compounds in water and their chemical stability, reduces volatility, protects against sunlight, masks unpleasant taste and odor, reduces side effects, increases bioavailability, thereby further increasing the therapeutic potential and efficacy of drugs [11 -17].

The high molecular weight, the presence of numerous donors and acceptors of hydrogen bonds in the structure, and pronounced hydrophilicity lead to low permeability of the considered oligosaccharides through biological membranes. Therefore, cyclodextrins in general show limited oral bioavailability (0.1 – 3.0%) in animals and humans, which makes them practically non-toxic. After oral administration, γ- CD is known to be readily metabolized in the small intestine by pancreatic α-amylase, while α- and β- CD are digested by bacterial microflora present in the colon [18, 19].

Mesalazine also has an antioxidant effect due to the association of its molecules with free radicals [20]. Currently, antioxidants are actively used in the treatment of gastric and duodenal ulcers [21, 22]. This suggests that MS and its complexes with cyclodextrins (MS ••• CD) may have a positive effect on the treatment of peptic ulcer.

The above facts indicate the promise of research on complex formation mesalazine with cyclodextrins. The proposed method of modifying MS can help in solving the problem of premature absorption and transport of mesalazine to damaged areas of the colonic mucosa. In addition, synthesized inclusion complexes may have improved physiological properties or other types of biological activity compared to the original components.

The purpose of this work is to study the possibility of complex formation mesalazine with cyclodextrins in aqueous solutions, determination of the composition and stability of the formed complexes, development of a procedure for the synthesis of inclusion complexes, development of a prototype (using an individual complex compound as an example) and study of its antiulcer activity *in vivo* .

2 Materials and methods

2.1 Reagents and equipment

In the experiment, we used α-, β-, and γ- cyclodextrins (AppliChem, Germany), 5-aminosalicylic acid (mesalazine) of the "pure" grade (JSC Khimreaktivsnab, Russia). The solvent was freshly distilled water. Electronic absorption spectra were recorded on a UV - 2401 PC spectrophotometer (Shimadzu, Japan) in thermostatic quartz cuvettes 1 cm thick relative to water.

2.2 Determination of compositions and stability constants of complex compounds

Complex compounds of mesalazine with α-, β-, and γ- cyclodextrins were obtained under equilibrium conditions at low concentrations of starting reagents (10^{-5}–10^{-4} mol/l). Complexation was studied spectrophotometrically in the wavelength range of 190–350 nm

at temperatures of 296–321 K. Under the experimental conditions, the UV spectra of MS aqueous solutions have two absorption bands with maxima at ~200 nm and ~298 nm. α-, β-, γ-Cyclodextrins do not give absorption peaks in the given wave range when light is transmitted, therefore all studies were performed at the absorption maxima of mesalazine.

The ratios of the components in the complexes were determined by the method of continuous changes [23, 24]. This method is based on determining the ratio of the concentrations of the interacting substances, which corresponds to the maximum yield of the formed complex compound. The extremum point on the curve indicates the maximum possible concentration of the complex, and its abscissa corresponds to the stoichiometric ratio of the reactants. To determine the composition of the complex compound, solutions of two components of the same molar concentration ($1 \cdot 10^{-4}$ mol/l), which were then mixed in ratios from 1:9 to 9:1, keeping the total volume of the solution unchanged. In this case, the total number of moles of both components in the solution always remained constant. After 1 hour, the optical densities of the solutions were measured. The comparison cuvette was filled with distilled water. Then, a graphical dependence of the change in optical density on the ratio of the concentrations of the components was plotted. The composition of the complex compound was found from the position of the extreme point on the obtained curve.

The stability constants of inclusion complexes of mesalazine and α-, β-, γ- cyclodextrins were determined by the saturation method [23, 24]. The essence of the method is to establish the dependence of the change in optical density on the concentration of one of the components at a constant concentration of the second component, and vice versa. The experiment was performed as follows: solutions of mesalazine and cyclodextrin were prepared with a concentration of $1 \cdot 10^{-4}$ mol/l. 2 ml of MS solution and from 0.5 to 8.0 ml of CD were poured into 10 volumetric flasks. The total volume of the mixture was then adjusted to 10 ml with water. After 1 hour, the optical density of the resulting solutions was measured. The results obtained were linearized in the coordinates of the corresponding equation (see below), from which the values of the stability constants of complex compounds were found from the ratio of the cutoffs to the tangents of the slope angles.

2.3 Method for the synthesis of complex compounds of mesalazine with cyclodextrins

Complex compounds MS···CD were synthesized by mixing equimolar amounts of mesalazine and cyclodextrins (α-, β- or γ- CD) in distilled water. The reaction mixture was stirred for four days at a temperature of 313 K, after which the water was removed by evaporation under reduced pressure. As a result, complex compounds were obtained in quantitative yield.

β-CD was developed, and its antiulcer effect was studied in comparison with the initial substances (MS and β-CD) and the reference drug (Omez).

2.4 Method for determining the antiulcer activity of the MS···β-CD inclusion complex

The antiulcer activity of the complex formed by mesalazine and β- cyclodextrin was studied in the medical chemistry group of the Ufa Institute of Chemistry, a separate structural subdivision of the Ufa Federal Research Center of the Russian Academy of Sciences.

The authors performed experiments in the spring on 30 outbred male rats weighing 350 g with a 24-hour food deprivation. Acute ulcers of the gastric mucosa were reproduced by a single intraperitoneal injection of indomethacin (Russia) at a dose of 20 mg/kg. The studied compounds (MS complex··· β-CD, mesalazine , β-cyclodextrin) were administered at a dose of 50 mg/kg, the reference drug Omez (India) at a dose of 20 mg/kg. Control animals received

distilled water in an equivalent volume. All compounds were administered orally 1 hour prior to indomethacin injection. 24 hours after the reproduction of an acute ulcer of the gastric mucosa, the animals were decapitated under ether anesthesia, opened, the stomachs were removed, and the average number of destructions (ulcers, erosions, hemorrhages) was calculated.

Statistical data processing was performed using the Statistica 7.0 package (StatSoft, United States of America). In the sample groups, the values of the median, lower and upper quartiles (Q25, Q75) were evaluated. The significance of differences was assessed using the Mann-Whitney test. Differences were considered statistically significant at $P < 0.05$.

3 Results and discussion

Checking the applicability of the Bouguer-Lambert-Beer law. At the first stage of research, the range of mesalazine concentrations in aqueous solutions was determined taking into account the Bouguer-Lambert-Beer law (1) [24] :

$$A = \varepsilon \cdot [MS] \cdot l, \qquad (1)$$

Where A is the optical density of the MS solution; ε is the molar absorption coefficient MS, l/(mol · cm); [MS] is the concentration of mesalazine in water, mol/l; l is the cell thickness, cm.

The calibration curve is shown in fig. 1. A clear linear relationship has been established between the optical density of the MS solution and its concentration, which does not exceed 10^{-4} mol/l. The molar absorption coefficient of mesalazine in water was calculated from the slope of the straight line:

$$\varepsilon = 3020 \pm 80 \text{ l/(mol} \cdot \text{cm)}.$$

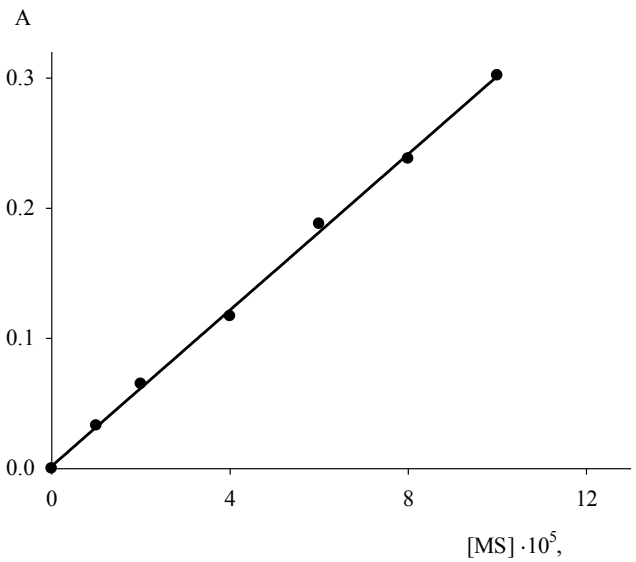

Fig. 1. Dependence of the optical density of the solution on the concentration of mesalazine 296 K, λ = 200 nm

Spectral detection of complex compounds, determination of their compositions. Comparison of ultraviolet (UV) spectra of aqueous solutions of mesalazine and products of its interaction with α-, β- and γ-cyclodextrins showed the following changes: 1-4 nm); 2) an increase in the intensities of the MS absorption band peaks ($\Delta A = 0.03 – 0.10$). The observed changes obviously indicate the appearance of intermolecular interactions between mesalazine and cyclodextrins and the formation of complex compounds of the "guest–host" type.

The presence of interactions in the reaction systems under study ("MS + α-CD + H_2O", "MS + β-CD + H_2O", "MS + γ - CD + H_2O") is also indicated by the data obtained by the method continuous changes [23, 24]. Thus, with an increase in the concentration of mesalazine, the curve of the dependence of the change in optical density (Δ A) on the ratio of the concentrations of MS and β- CD , presented as an example in Fig. 2 goes through a maximum. Similar dependences were established for mesalazine complexes with α- and γ-cyclodextrins. This fact confirms the existence of interactions between MS and the considered oligosaccharides, otherwise the above dependence would have been linear.

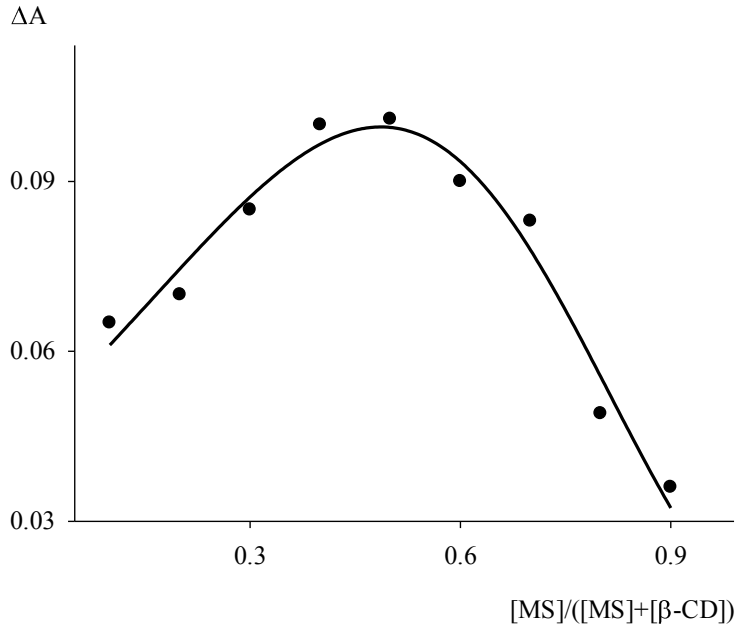

Fig. 2. Graphical dependence of the change in the optical density of solutions on the ratio of the concentrations of mesalazine and β- cyclodextrin ; 296 K, [MS] + [β - CD] = $1 \cdot 10^{-4}$ mol /l

The results obtained by the method of continuous changes also made it possible to determine the ratio between the stoichiometric coefficients of the reactants. According to this method, the initial ratio of the components in the system, at which the concentration of the formed complex compound is maximum, is equal to their ratio in the complex. Analysis of fig. 2 indicates that mesalazine and β- cyclodextrin interact in a molar ratio of 1:1. For complex compounds of MS with other CDs (α- CD , γ- CD), it has been established that the stoichiometric ratio " guest : master" is also equal to 1:1.

Determination of the stability constants of complex compounds. The stability constants of complex compounds were found using the saturation method [23, 24] in the temperature range of 296–321 K. According to this method, the spectral changes in the studied reaction systems are described by equation (2):

$$\frac{[MS]}{\Delta A} = \frac{1}{\Delta\varepsilon} + \frac{1}{\Delta\varepsilon \cdot K \cdot [CD]}, \qquad (2)$$

where [MS] is the concentration of mesalazine, mol/l; Δ A is the difference between the optical densities of solutions of the complex compound and mesalazine ; Δε is the difference between the molar absorption coefficients of the complex compound and mesalazine , l/(mol · cm); K is the stability constant of the complex compound, l/mol; [CD] – concentration of cyclodextrin , mol/l.

To determine K, plots of [MS]/ΔA versus 1/[CD] were plotted, from which the stability constants of complex compounds were found from the ratio of cutoffs to slope tangents. The obtained values of K are presented in table 1.

Table 1. Temperature dependences of stability constants of complex compounds formed by mesalazine and α-, β-, γ- cyclodextrins

T, K	$K \cdot 10^{-3}$, l/mol		
	MS ··· α-CD	MS ··· β-CD	MS ··· γ-CD
296	10.1 ± 1.1	64.1 ± 7.1	33.2 ± 3.8
301	5.1 ±0.5	18.7 ± 2.4	17.9 ±2.1
306	1.6 ±0.2	10.5 ± 1.3	9.4 ±1.1
311	1.0 ±0.1	2.9 ± 0.3	6.7 ±0.8
316	0.40 ±0.05	1.1 ± 0.1	3.4 ±0.5
321	0.30 ±0.03	-	1.8 ±0.2

Analysis of the table shows that in dilute aqueous solutions, mesalazine forms relatively strong inclusion complexes with cyclodextrins ($K \sim 10^2 \div 10^4$ l/mol). From Table 1 we can conclude that the stability constants of complex compounds decrease with increasing temperature.

Determination of thermodynamic parameters of complexation. Table 1 data were used to calculate the standard thermodynamic parameters (enthalpy change - ΔH°, entropy change - ΔS°) of reactions between mesalazine and cyclodextrins in an aqueous medium. For this, equation (3) [25] was used:

$$ln\, K = \frac{\Delta S^\circ}{R} - \frac{\Delta H^\circ}{R} \cdot \frac{1}{T}, \qquad (3)$$

linking the thermodynamic parameters with the stability constant of the resulting complex compound.

After processing the data in Table 1 in the coordinates of equation (3) (Fig. 3), the changes in the enthalpies and entropies of the reactions of complex formation were calculated mesalazine with cyclodextrins in water (Table 2). The standard value of the Gibbs energy change was found using equation (4) [25]:

$$\Delta G^\circ = \Delta H^\circ - T \cdot \Delta S^\circ. \qquad (4)$$

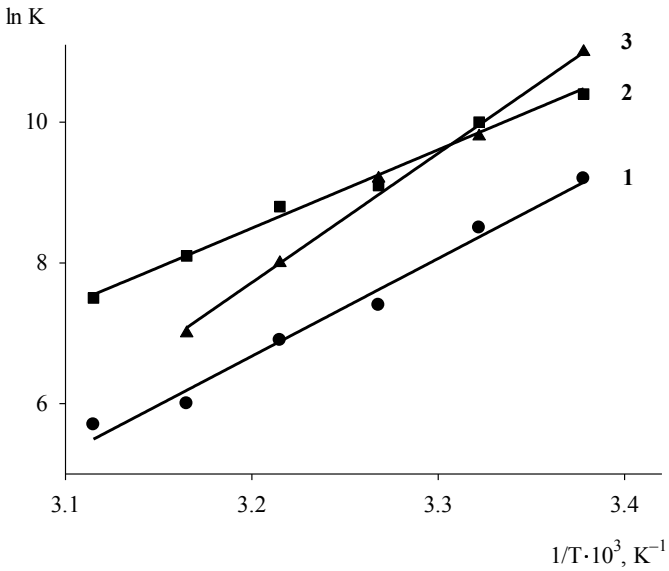

Fig. 3. Linearization of the temperature dependences of the stability constants of the complex compounds formed by mesalazine and α- (1), β- (2), γ- (3) cyclodextrins, in the coordinates of equation (3)

Table 2. Standard thermodynamic parameters of reactions of formation of complex compounds of mesalazine with α-, β-, γ- cyclodextrins

complex connection	Δ G °(296 K), kJ/mol	ΔH _° · 10 $^{-2}$, kJ/mol	ΔS _° · 10 $^{-2}$, J/(mol · TO)
MS ··· α- CD	– 25 ± 3	– 1.2 ± 0.2	– 3.2 ± 0.5
MS ··· β- CD	– 33 ± 5	–1.6 ± 0.2	– 4.3 ± 0.5
MS ··· γ- CD	– 2 5 ± 3	– 0.9 ± 0.1	– 2.2 ± 0.2

Table 2 shows that all standard thermodynamic parameters (Δ G °, Δ H °and Δ S °) have a negative sign. The data obtained indicate the spontaneous occurrence of complex formation reactions in the studied temperature range (296 – 321 K), their exothermicity and a decrease in the number of particles due to the association of "guest" molecules with "host" molecules when immersed in the internal cavity of cyclodextrins .

Development of a methodology for the synthesis of complex compounds, development of one of the complexes. The obtained information on the composition, stability constants, and thermodynamic parameters were used in the development of a procedure for the synthesis of MS ··· CD complexes (see *Materials and methods*), which consists in mixing equimolar amounts of the starting substances (MS and CD) in water, maintaining the reaction mixtures at temperatures close to the physiological maximum for a certain time and subsequent removal of the solvent.

For biological testing, based on the proposed method, a complex of mesalazine with β-cyclodextrin (MS···β-CD) was synthesized. This complex was not chosen by chance. It is β-CD (or its derivative) that is most often considered as the most promising drug carrier due to its safety (allowed as a food additive E 495 in many countries, including Russia), availability and low price. In addition, the inner diameter of the β- CD ring , equal to 0.7–0.8 nm , corresponds to the size of most molecules of low molecular weight biologically active compounds.

Study of the antiulcer activity of the complex compound of mesalazine with β-cyclodextrin. The antiulcer activity of the inclusion complex MS ··· β- CD was studied in the model of acute indomethacin ulcer. It is known [26, 27] that intraperitoneal administration of indomethacin leads to a decrease in the concentration of prostaglandins, especially PGE_2 , in the gastric mucosa (GM) and a decrease in all protective functions, a decrease in the synthesis of mucus and bicarbonates, a deterioration in blood flow, and an increase in acid secretion. In this case, the accumulation of hydrogen peroxide and active oxygen radicals in the ischemic areas of the coolant. Strengthening free radical processes leads to an increase in lipid peroxidation of membranes and a violation of the integrity of the gastric mucosa.

The researchers used Omez (omeprazole) as a reference drug. Its main property is the inhibition of the acid-forming function of the stomach by inhibiting the intracellular enzyme H^+/K^+-ATPhase (often called the cellular pump, or proton pump) and thereby the production of hydrochloric acid [28]. The effectiveness of omeprazole is defined as a standard of antisecretory response: it significantly affects the rate of relief of symptoms and scarring of the ulcer, and also ensures maximum safety of treatment. This drug can now be considered the standard in the treatment of peptic ulcer [29].

A day after the administration of indomethacin, the animals of the control and experimental groups were observed to have ulcers (from pinpoint to large), erosions and hemorrhages in the gastric mucosa. One should note that ulcers with a black bottom predominated, formed as a result of contact of blood with hydrochloric acid of the stomach (hydrochloric hematin).

The studied compounds (mesalazine , β- cyclodextrin , MS···**β-CD** complex, and reference drug Omez) were administered orally to the animals 1 hour before the injection of indomethacin . The results of the study of the antiulcer action of these compounds and the reference drug are given in table 3.

Table 3. Influence of the complex compound of mesalazine with β- cyclodextrin and reference drugs on the number of destructions of the gastric mucosa of rats (the number of animals in the group N = 6)

Group number	Compound	Dose, mg/kg	Number of destructions
1	MS ··· β- CD	50	9.0*
2	MS	50	15.0*
3	β - CD	50	15.5*
4	Omez	20	8.5*
5	Control	-	20.5*

* significant relative to control, $P < 0.05$

The table shows that the introduction of the studied compounds leads to a decrease in the number of destructions of the mucosa of rats. The initial substances, mesalazine and β-cyclodextrin, reduced the number of destructions compared to the control by 1.37 and 1.32 times, respectively. Omez reduced the number of destructions by 2.4 times, and the studied complex MS···β-CD – by 2.3 times compared with the control.

Thus, in the model of ulcers caused by indomethacin, the complex of mesalazine with β-cyclodextrin at a dose of 50 mg/kg showed a more pronounced antiulcer activity compared to the initial substances (MS and β- CD) and approximately the same activity as that of the reference drug Omez. It is important to note that to obtain the complex compound MS··· β-CD, the starting materials were mixed in an equimolar ratio, from which it follows that the amount of mesalazine in the complex is only 12% wt., the remaining 88% wt. account for β-cyclodextrin.

Determination of the molar absorption coefficient of the complex compound mesalazine with β-cyclodextrin. The dependence of the optical density (A) of aqueous solutions of the synthesized inclusion complex MS···β-CD on its concentration ([MS ··· β- CD]) was studied. From the slope of the straight line A = f ([MS ··· β- CD]), the molar absorption coefficient of the complex compound of mesalazine with β- cyclodextrin in aqueous solution:

$$\varepsilon = 3420 \pm 160 \; l/(mol \cdot cm).$$

The obtained value of ε coincides (within the error of its determination) with the value of the molar absorption coefficient ε = 3280 l/(mol · cm) found by equation (2). One should note that the discrepancy between the given values is only 4%. This fact is an additional confirmation of the identity of MS complexes with β-CD synthesized according to the method proposed above and obtained in a dilute aqueous solution.

4 Conclusion

The authors found that mesalazine and α-, β -, γ- cyclodextrins in aqueous solutions interact in a molar ratio of 1:1 with the formation of relatively stable inclusion complexes (K ~ $10^2 \div 10^4$ l/mol). The dependence of stability constants of complex compounds MS···α - CD, MS···β - CD and MS···γ- CD on temperature in the range of 296 – 321 K was studied, and the thermodynamic parameters of complex formation were calculated. Based on the data obtained, the authors developed a method for the synthesis of complex compounds of mesalazine with cyclodextrins and developed a prototype of one of the complexes (MS···β-CD) and studied its antiulcer effect. Pharmacological studies have shown that the MS···β-CD complex exhibits a more pronounced antiulcer activity compared to the parent substances (MS and β-CD) and is comparable to Omez used as a reference drug. Thus, the results obtained in this work indicate the possibility of the formation of complex compounds of mesalazine with α -, β -, γ - cyclodextrins, which can subsequently be used in the preparation of medical anti-inflammatory drugs with directed action or become the basis of drugs for the treatment of peptic ulcer of the stomach and duodenum.

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