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A METHOD FOR THE OBTAINING OF INCREASED VISCOSITY EYE DROPS CONTAINING AMIKACIN

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Abstract: A method for the obtaining of increased viscosity (3.6-52.5 cP) eye drops, containing amikacin–aminoglycoside antibiotic and as increasing viscosity agents: polivinyl alkohol, hydroxyethylcellulose or sodium hyaluronate was elaborated. Physicochemical and biological properties of these eye drops were determined.

Keywords: eye drops; amikacin sulphate; pharmaceutical interaction of amikacin sulphate

Water solutions of eye drops belong to the most often used ophthalmic drugs. Such solutions are applied to the cornea or into the conjunctiva sack in the amount of 1–2 drops, twice to four times a day. Because of the lacrimal system dynamics, eye drops are easily washed away with lacrimal fluid and removed from the eye. Therefore, the time of the direct contact of the fluid with the eye surface is short, and the amount of the drug absorbed constitutes only a part of the recommended dose. Hence, preparations of this form require often reapplication if the therapy is to be successful.

Prolongation of the time when the therapeutic substance remains in the application spot can be achieved by applying higher viscosity systems, which enable longer contact with the eye surface and thus increased bioavailability of the drug. The most often used substances improving viscosity of eye drops preparations are: methylcellulose 500 – 4000 mPs, hydroxypropylmethylcellulose, hydroxyethylcellulose (1a), sodium carboxymethylcellulose, polyvinyl alcohol (1b) and polyacrylic acids (Carbopol 934, 940, 971, 974 P and 980 NF) (1c).

Eye drop preparations containing antibiotics are known, including widely applied in therapy aminoglycoside antibiotics, such as tobramicin or amikacin (2, 3). The latter shows antibacterial effect against both Gram–positive and Gram–negative microorganisms. Action range includes bacterial strains *Pseudomonas sp., Escherichia coli*, indolo–positive and indolo–negative *Proteus sp*., as well as *Providentia sp., Klebsiella, Enterobacter, Seratia sp*. and *Acinetobacter sp*. Among Gram–positive microorganisms, the strongest action is registered against the bacterial strains of *Staphylococcus aureus* and *Streptococcus epidermidis*, and also against methicillin–resistant and β–lactamase–positive bacterial strains. The minimal inhibiting concentration (MIC) against most of the bacterial strains is 2–4 μg/mL, and for some Gram–positive strains, the MIC values are even lower. An important advantage of amikacin is its effectiveness against many pathogenic bacterial strains tobramycin– and gentamycin–resistant (4).

The commercial preparation of amikacin eye drops are known as Biodacyna ophthalmicum 0.3%, produced by IBA–Bioton company. The preparation is used in the treatment of bacterial conjunctiva, cornea affections, in eyelid edges inflammation and stye. This drug is also used preventively before eye surgery treatment. Biodacyna ophthalmicum 0.3% should be applied into conjunctiva sack 3–4 times a day, because of the short time of remaining on the eye surface (5).

The aim of our research was to elaborate a new increased viscosity ophthalmic form, containing amikacin, and to examine its physicochemical and biological properties.

EXPERIMENTAL

Reagents

Amikacin disulphate (Pharmatex Italia S. R. L.) of purity according to the requirements of Pharmacopoeia (6a); sodium hialuronate of molecular weight 1.34–1.5 MDa and 1.64 MDa (Contipro C Co.); polyvinyl alcohol PA–18 GP (Shin Etsu) and Mowiol 40–88 (Fluka); hydroxyethylcel-

lulose–Natrosol, (Herkules); Carbomer 980 NF (BF Goodrich); Gellan Gum–Kelcogel F (CP Kelco); cetrimonium bromide (Rona Care™, Merck); benzalkonium chloride (Fluka); benzalkonium bromide (Fluka); thiomersal (Sigma); sodium laurylosulphate (Merck); Tris [(hydroxymethyl)–aminomethane] (Merck); dihydrate disodium hydrogen phosphate (AppliChem); hydrate sodium dihydrogen phosphate (POCH SA); boric acid (Merck); decahydrate disodium tetraborate (Merck).

All appplied reagents, except Gellan gum, were of pharmacopoeial or pharmaceutical purity.

Determination of compatibility of amikacin solution and viscosity increasing – as well as antibacterial preservative agents

In order to check the compatibility of amikacin solutions and additives increasing viscosity as well as antibacterial preservative agents some of these compounds were examined. The results are presented in Table 1.

Based on the obtained results, the following substances have been selected for further examination as viscosity improving means: polyvinyl alcohol, hydroxyethylcellulose and sodium hyaluronate and benzalkonium chloride and bromide as the preservative agents.

Preparation of increased viscosity eye drops containing amikacin

Obtaining of eye drops containing polyvinyl alcohol (Preparations **1** and **2**)

60 g of polyvinyl alcohol PA–18GP or 80 g of polyvinyl alcohol Mowiol 40–80 was added during vigorous stirring to 3.6 L of injection water. The obtained suspension was heated to a temperature of 75–80^{o}C and stirred until dissolution for 1 hour. Then it was cooled down to room temperature and to the obtained solution were added successively: 57.52 g of dihydrate disodium hydrogen phosphate, 11.84 g of hydrate sodium dihydrogen phosphate and 0.44 g of benzalkonium chloride (calculated for the anhydrous substance), 8.0 g of sodium chloride and amikacin disulphate containing 12.0 amikacin (calculated for the dried basis). The whole of the obtained solution was completed with injection water to a weight of 4.07–4.08 kg, and then filtered through a set of filters containing a prefilter 0.3 μm and a sterilizating filter 0.22 μm. The filtrate was proportioned into polyethylene containers of 5.0 mL volume each, using an automatic proportioner, then the containers were equipped with droppers, protective caps and labels.

Obtaining of eye drops containing hydroxyethylcellulose (Preparation **3**)

18.4 g of sodium chloride, 0.44 g of benzalkonium chloride (calculated for the anhydrous substance) and amikacin disulphate containing 12.0 g of amikacin (calculated for the dried basis) were added successively to 3.6 L of phosphate buffer of pH = 7.2–7.3, containing in this volume 71.9 g of dihydrate disodium hydrogen phosphate and 14.8 g of hydrate sodium dihydrogen phosphate. Then 22.0 g of hydroxyethylcellulose was added and stirred, until the clear solution was obtained, which then was completed with previously prepared phosphate buffer, up to a weight of 4.090-4.092 kg. The obtained solutions were filtered and administered into polyethylene containers in the conditions given in Preparations 1 and 2.

Obtaining of eye drops of viscosity 16-23 cP, containing sodium hyaluronate (Preparations **4** and **5**)

0.44 g of benzalkonium bromide (calculated for the anhydrous substance), 8.0 g of sodium chloride, 8.56 g of sodium hyaluronate of molecular weight 1.34–1.5 MDa or 1.64 MDa and amikacin disulphate containing 12.0 g of amikacin (calculated for the dried basis) were added successively to 3.6 L of phosphate buffer of pH = 7.2–7.3 containing in this volume 57.52 g of dihydrate disodium hydrogen phosphate and 11.84 g of hydrate sodium dihydrogen phosphate. It was stirred until clear solution was obtained, and then completed with injection water, up to a volume of 4.0 L. The obtained solution was filtered and administered into the polyethylene containers, in the conditions given in Preparations 1 and 2.

Obtaining of eye drops of viscosity 52.2 cP, containing sodium hyaluronate (Preparation **6**)

0.44 g of benzalkonium bromide (calculated for the anhydrous substance), 8.00 g of sodium chloride, 17.12 g of sodium hyaluronate of molecular weight 1.34 – 1.50 MDa and amikacin disulphate containing 12.0 g of amikacin (calculated for the dried basis) were added successively to 3.6 L of phosphate buffer of pH = 7.2 – 7.3, containing in this volume 57.52 g of dihydrate disodium hydrogen phosphate and 11.84 g of hydrate sodium dihydrogen phosphate. It was stirred until clear solution was obtained, and then completed with injection water, up to a volume of 4.0 L. The obtained solution was filtered and administered into the polyethylene containers, in the conditions given in Preparations **1** and **2**.

Table 1. Compatibility of amikacin buffer solution with viscosity increasing and antibacterial preservative agents

No.	Additives present in amikacin buffer solution, containing 3 mg/mL of amikacin (calculated on the dried basis)					Clarity
	Viscosity increasing agent	Concentration (%)	Preservative antibacterial agent	Concentration (%)	Buffer	
1	sodium hyaluronate	0.3	benzalkonium bromide	0.1	phosphate	Clear
2	”	0.2	benzalkonium chloride	0.1	”	”
3	”	”	”	”	”	”
4	”	”	”	”	contg. Tris	”
5	”	”	”	”	borate	”
6	”	”	thiomersal	”	phosphate	”
7	”	”	cetriminium bromide	”	”	opalescence
8	polyvinyl alcohol	1.5	benzalkonium chloride	”	”	clear
9	”	”	thiomersal	”	”	”
10	”	”	cetriminium bromide	”	”	”
11	hydroxy-ethylcellulose	0.55	benzalkonium chloride	”	”	”
12	”	”	”	”	borate	”
13	”	”	thiomersal	”	phosphate	”
14	”	”	cetriminium bromide	”	”	”
15	Carbomer (*980 NF*)	0.5	benzalkonium chloride	”	”	precipitation
16	”	”	”	”	borate	”
17	”	”	”	”	contg. Tris	”
18	”	0.2	”	”	phosphate	”
19	gellan gum (*Kelcogel F*)	0.25	benzalkonium bromide	”	phosphate	opalescence
20	”	”	”	”	borate	”

The physicochemical properties of the obtained eye drops (Preparations **1–6**) are presented in Table 2.

METHODS

Apparatus

pH–meter (Methrom 691), viscosimeter (Brookfield Engineering Labs. Inc.), osmometer (Knauer), Fishers Apparatus to measurements inhibition zone, Brookfield Digital Rheometer DV-III+; UV 520 UV–Visible Spectrometer (Unicam), osmometer Marcel OS 3000 (Marcel), Climatic Cabinets Rumed 4201 and Rumed 4301.

Methods for the analysis of obtained eye drops

Microbiological assay of amikacin in the solutions of eye drops by diffusion method

The potency of an amikacin disulphate was estimated by comparing the inhibition of growth of sensitive microorganisms produced by known concentrations of the antibiotic to be examined and a reference substance. The antimicrobial activity of amikacin in the drops was determined by the cylinder-plate method according to PPh VI using BR 1 medium (phosphate buffer pH = 6.0), test microbial strain *Bacillus subtilis* ATCC 6633 (1 mL/300 mL of medium) and amikacin disulphate in phosphate buffer pH = 6.0 (7). The method was validated.

Determination of physicochemical properties of eye drops

pH was measured using a pH-meter Methrom 691 (6b), the viscosity was determined using a rota-

Table 2. Physicochemical parameters of obtaining eye drops containing of amikacin

Preparation No.	Amikacin concentration	Liquid content in the container	pH	Colour	Clarity	Benzalkonium	Viscosity chloride concentration	Osmolality
	(mg/mL)	(mL)		$E_{415nm, 5cm}$	$E_{600nm, 5cm}$	(mg/mL)	(cP)	(mOsm/kg)
1	3.0	5.0	7.2	0.02	0.002	0.1	3.6	330
2	3.0	5.2	7.1	0.02	0.004	0.1	7.1	298
3	3.0	5.0	7.2	0.01	0.002	0.11	7.2	294
4	3.0	5.1	7.3	0.021	0.008	0.1	16.2	296
5	3.0	5.1	7.2	0.018	0.006	0.1	22.9	302
6	3.0	5.1	7.2	0.025	0.01	0.11	52.5	320

Table 3. Long-term stability study (25˚C±2˚C, 60%±5% RH); amikacin 3 mg/mL (Preparation **1**)

Test	Limit	Time (months)				
		0	3	6	9	12
Appearance	Colourless, clear solution	Complies	Complies	Complies	Complies	Complies
pH	6.0-8.0	7.2	7.1	7.0	7.0	6.9
Assay of amikacin	2.7 – 3.3 mg/mL	3.0	2.9	3.1	3.0	3.0
Clarity [$E_{5\ cm,\ \lambda=600\ nm}$]	Not more than 0.050	0.004	0.005	0.004	0.008	0.010
Viscosity	3.0 – 4.0 cP	3.5	3.6	3.6	3.6	3.6
Osmolality	281-334 mOsm/kg	315	316	314	314	318
Benzalkonium chloride content	0.08 – 0.14 mg/mL	0.14	0.12	0.14	0.14	0.14
Efficacy of antimicrobial preservative		Complies				

ting viscosimeter (6c). The clarity was determined using a spectrometer and the measurement of the absorbance was carried out at a wavelength 600 nm in a 5 cm cell (6d).

Osmotic pressure was determined using an osmometer (6e).

Determination of assay of preservatives (benzalkonium chloride and benzalkonium bromide)

The assay of the preservatives was determined by the titration method. The method is based upon the quantitative reaction of benzalkonium chloride or benzalkonium bromide with sodium laurylsulphate in the presence of a base with sufficient buffering capacity. The method was validated (unpublished).

Test of preservation efficiency of benzalkonium chloride and benzalkonium bromide

The antimicrobial efficiency of benzalkonium chloride or benzalkonium bromide was determined by the preservation assay according to Ph. Eur, using reference microbial strains: *Pseudomonas aeruginosa ATCC 9027, Staphylococcus aureus ATCC 6538, Candida albicans ATCC 10231 and Aspergillus niger ATCC 16404* (6f). The method was validated.

Stability studies

Stability tests on the eye drops was performed according to the International Conference on Harmonisation (ICH) requirements (8). During a long term stability study the samples were kept at 25 ±2˚C/60% relative humidity (RH) and tested at the time of release and intervals of every three months for the first 12 months and every six months during the second year. Prior to this the storage conditions were 22 ±3˚C and ambient humidity.

The ICH Guideline suggests that the minimum acceptable stability data for product registration submissions is 12 months for a long–term testing at 25±2˚C/60% RH and 6 months for intermediate conditions at 30 ±2˚C/60% RH.

Table 4. Intermediate conditions stability study (30°C±2°C, 60%±5% RH); amikacin 3 mg/ml (Preparation **1**)

Test	Limit	Time (months)				
		0	3	6	9	12
Appearance	Colourless, clear solution	Complies	Complies	Complies	Complies	Complies
pH	6.0-8.0	7.2	7.0	6.9	6.9	6.8
Assay of amikacin	2.7 – 3.3 mg/mL	3.0	3.0	3.0	3.0	3.0
Clarity [$E_{5\ cm,\ \lambda=600\ nm}$]	Not more than 0.050	0.004	0.006	0.005	0.017	0.026
Viscosity	3.0 – 4.0 cP	3.5	3.6	3.7	3.6	3.6
Osmolality	281-334 mOsm/kg	315	313	312	315	310
Benzalkonium chloride content	0.08 – 0.14 mg/mL	0.14	0.12	0.14	0.14	0.14
Efficacy of antimicrobial preservative		Complies				

Table 5. Long-term stability study (25°C±2°C, 60%±5% RH); amikacin 3 mg/ml (Preparation **2**)

Test	Limit	Time (months)				
		0	3	6	9	12
Appearance	Colourless, clear solution	Complies	Complies	Complies	Complies	Complies
pH	6.0-8.0	7.2	7.1	7.0	6.9	6.9
Assay of amikacin	2.7 – 3.3 mg/mL	3.0	2.9	2.9	3.0	2.9
Clarity [$E_{5\ cm,\ \lambda=600\ nm}$]	Not more than 0.050	0.001	0.009	0.006	0.007	0.016
Viscosity	6.0 – 8.0 cP	7.0	7.3	7.2	6.9	6.8
Osmolality	281-334 mOsm/kg	325	322	323	327	326
Benzalkonium chloride content	0.08 – 0.14 mg/mL	0.12	0.14	0.09	0.12	0.13

Stability testing were completed at 30/60% RH. The long-term testing will be continued for a sufficient period of time to cover all appropriate retest periods. The batches of the drops were stored in polyethylene containers that are similar to the definitive pack.

The stability study results are summarised in Tables 3–6.

Determination of amikacin level in lacrimal liquid

Ocular pharmacokinetic examination of Preparation 5 and Biodacyna Ophtalmicum 0.3% has been conducted on 20 New Zealand–breed rabbits (4 rabbit per one time point). The animals have been administered simultaneously two drops: Biodacyna Ophthalmicum 0.3% into the right eyes and Preparation 5 into the left ones. After 0.5, 1, 2, 4 and 10 h, aliquots for microbiological examination have been sampled, placing 6.0 mm Whatman tissue paper discs on the eye formix inferior, and then closing the eye for about 10 s and removing the discs with the forceps. Amikacin concentration has been tested microbiologically against the bacterial strain *Bacillus subtilis ATCC 6633*. Prior to the microbiological test the lacrimal fluid of each rabbit had been tested for absence of bacteria growth reducing substances.

DISCUSSION

The method for the preparation of increased viscosity of eye drops, containing amikacin sulphate and as viscosity improving means polyvinyl alcohol, hydroxyethylcellulose and sodium hyaluronate was elaborated. Polyvinyl alcohols and derivatives of hydroxyalkylcellulose are encountered in ophthalmic preparations, however sodium hyaluronate, being a natural glycosoaminoglycan, occurs rather relatively seldom. The presence of sodium hyalurona-

Table 6. Intermediate conditions stability study (30°C±2°C, 60%±5% RH); amikacin 3 mg/ml (Preparation **2**)

Test	Limit	Time (months)				
		0	3	6	9	12
Appearance	Colourless, clear solution	Complies	Complies	Complies	Complies	Complies
pH	6.0-8.0	7.2	7.0	6.9	6.8	6.7
Assay of amikacin	2.7 – 3.3 mg/mL	3.0	3.0	3.0	2.9	2.9
Clarity [$E_{5\ cm,\ \lambda=600\ nm}$]	Not more than 0.050	0.001	0.008	0.006	0.015	0.021
Viscosity	6.0 – 8.0 cP	7.0	7.2	7.0	6.9	6.8
Osmolality	281-334 mOsm/kg	325	328	324	327	320
Benzalkonium chloride content	0.08 – 0.14 mg/mL	0.12	0.14	0.09	0.10	0.13

te in the ophthalmic preparation reduces intensity of local undesirable actions, including irritating action caused by the preservative agent.

Addition of this compound at a concentration of 0.1 – 0.5% demonstrates protective action on the cornea epithelium, without any changes in the pharmacodynamical properties of active substance, including its bioavailability. Owing to the content in its ingredients, the ophthalmic preparation gains more tolerance, and because of improved viscosity, better adhesion to the cornea surface and prolonged contact of the eye surface with active substance. An additional advantage of sodium hyaluronate is the fact that it eliminates the erosion of eye tissue caused by the condition of inflammation. This, in connection with antibacterial action of amikacin, may result in faster therapeutic effects.

The obtained Preparations of the wide range of viscosity 3.6–52.5 cP are stable under accelerated storage conditions over a testing period of 12 months, for a long-term testing respectively. The results presented in Tables 3–6 show that the slight fluctuation in any of the parameters tested during study time was raised by the analytical test procedure and was not considered to be critical. All results comply well specifications. No significant change was observed during storage of the eye drops. Stability of Preparations obtained according to the methods presented in this work are similar to the stability of Biodacyna Ophtalmicum0.3%.

The examination of sterility performed after the mentioned period demonstrated that the listed preparations preserved sterility (Table 5).

Comparative examination of Biodacyna ophthalmicum 0.3% and Preparation 5 of viscosity 22.9 cP, conducted on the rabbits demonstrated that, in case of applying Preparation 5, the therapeutic value of MIC for amikacin had been preserved still after 10 h after the application time, while for Biodacyna Ophtalmicum 0.3% MIC values just after 4 h had been very low.

Moreover, the results of the examination, conducted in the conditions mentioned above, where the levels of amikacin obtained in lacrimal fluid of rabbits after applying Biodacyna ophthalmicum 0.3% and Preparation 5 with the declined level of the antibiotic to 1.8 mg/mL have been compared, show similar levels of amikacin, obtained at a time of 0.5–2.0 h after the application time of both substances. The remains of the therapeutic action within the eye have also been observed, even up to 12 h after the application of Preparation 5 with the declined level of the antibiotic to 1.8 mg/mL which was much longer than after applying the commercial preparation.

The correct dosage of Preparation 5 and its frequency of application depends on the type of disease and doctor's recommendations, but biological data presented in the article suggest the possibility of applying Preparation 5 only twice a day, instead of 3–4 times daily, as in case of Biodacyna Ophtalmicum 0.3%. It is also connected with the decline of amikacin unitary dose administered into the eye, described as therapeutic dose.

The preliminary comparative examination *in vivo* of the obtained Preparations 1–6 show that the drops with polyvinyl alcohols and with hydroxyethylcellulose (Preparations 1–3) possess similar therapeutic effectiveness, but preparations made on the basis of sodium hyaluronate (Preparations 4–6) are characterised with significantly better effectiveness. This effectiveness in all cases is higher than that of Biodacyna ophtalmicum 0.3%.

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