Acta Poloniae Pharmaceutica – Drug Research, Vol. 63 No. 3 pp. 225–234, 2006 ISSN 0001-6837
Polish Pharmaceutical Society

PHARMACEUTICAL TECHNOLOGY

INFLUENCE OF ADDITIVES AND STORAGE TEMPERATURE ON PHYSICOCHEMICAL AND MICROBIOLOGICAL PROPERTIES OF EYE DROPS CONTAINING CEFAZOLIN.

ANNA KODYM [1], TOMASZ ZAWISZA [2], KAMILA BUŹKA [2] and HELENA KUKUŁA [3]
[1] Department of Drug Form Technology, Nicolaus Copernicus University Collegium Medicum in Bydgoszcz,
[2] Department of Drug Form Technology,
[3] Department of Pharmaceutical Bacteriology; K. Marcinkowski University of Medical Sciences in Poznań

Abstract: The purpose of the studies was to choose additives for eye drops containing cefazolin and the assessment of the influence of used additives and the storage temperature on the physicochemical properties and the stability of the eye drops. The drops were 1% sterile solutions of cefazolin in citrate buffer of pH 6.15-6.20. The drops were preserved with 0.002% thiomersal or 0.001% phenylmercuric borate mixed with 0.4% β-phenylethyl alcohol. Viscosity of the eye drops was increased using polyvinyl alcohol (PVAL). The pharmaceutical compatibility test of selected additives with cefazolin showed the pharmaceutical interaction of 1% solution of cefazolin with higher than 0.003% concentration of thiomersal, 0.005% benzalkonium chloride and 0.01% chlorhexidine diacetate. The drops, protected from light, were stored at the temperature of 4°C and 20°C. As the criteria of the qualitative assessment of freshly prepared drops and during their storage, the following properties were considered: organoleptic analysis, sterility, pH, osmotic pressure, density, viscosity, antimicrobial activity of cefazolin and preservation efficiency of thiomersal and phenylmercuric borate in the eye drops. The studies showed that the storage temperature did not influence the physicochemical properties of the drops or the antimicrobial activity of cefazolin in the drops, which was not influenced by the used additives either. After 30 days of storage at both temperatures, cefazolin in the eye drops retained 100% of its initial activity. Phenylmercuric borate, whose antimicrobial activity in the eye drops was compatible with the preservation assay cited in the Polish Pharmacopoeia (PPh V), can be used to preserve the drops containing cefazolin.

Keywords: cefazolin, eye drops, pharmaceutical interactions of cefazolin.

Cefazolin is a cephalosporic antibiotic of generation I, which works efficiently against Gram-positive cocci i.e. staphylococci *Staphylococcus aureus* (excluding *MRSA*) and *Staphylococcus epidermidis,* and streptococci *Streptococcus pneumoniae*, *Streptococcus pyogenes* and *Streptococcus viridans*. Cefazolin shows also antimicrobial activity towards some Gram-negative bacteria such as *Klebsiella pneumoniae* and *Escherichia coli* and towards anaerobic cocci and bacilli, which are sensitive to penicillin (1).

Eye drops at the concentration of 1-5% are used in the treatment of inflammations of eyelids, ulcerous inflammations of eyelids, conjunctivitis, neonatal conjunctivitis and pyogenic keratitis (2). In case of serious infections of the eye such as intraocular inflammation, inflammation of connective tissue of the eye socket, and ulcerous inflammation of the corneal epithelium or with corneal perforation, cefazolin is used systemically combined with the topical treatment in form of periocular injections or injections into vitrous body and in form of 5% eye drops (2). Cefazolin in the topical and general treatment of serious eye infections is mixed with other antibiotics e.g. gentamicin (3), tobramycin (4, 5) and ofloxacin (6).

There are no commercial forms of eye drops containing cefazolin available on pharmaceutical market. They are prepared *ex tempore* by hospital pharmacies for the patients of ophthalmic wards. Commercial preparations of artificial tears (4, 7, 8), buffer solutions such as acetate buffer of pH 4.5 and 5.7 and phosphate buffer of pH 7.5, solutions of sodium chloride (8, 9) and 1% glycerol (9) were used as cefazolin solvents in the eye drops. Stability of cefazolin in the eye drops is dependent on the storage temperature and pH (8, 9). It is more stable in solutions of acidic pH, e.g. pH 4.5 and 5.7 in comparison with the solution of pH 7.5 (8). It retained 93% of its initial contents in the drops after 42 days of storage at the temperature of 4°C, while after the same time in the drops stored at the room temperature only 50% of cefazolin was left (9).The purpose of the studies was the choice of necessary additives for the eye

drops containing cefazolin and the assessment of the influence of the used additives and the storage temperature on the physicochemical properties and antimicrobial activity of cefazolin in the drops.

EXPERIMENTAL

Materials

Kefzol® (Cefazolinum natricum), Eli Lilly Italia, vials a 1.0 g, dry substance for intramuscular and intravenous injections; eye drops containing cefazolin, prepared under aseptic conditions according to the formulary composition showed in Table 1; sterile solutions: cefazolin, citrate buffers, polyvinyl alcohol (PVAL), preservatives: benzalkonium chloride, thiomersal, phenylmercuric borate and chlorhexidine diacetate.

Reagents

Citric acid monohydrate, sodium citrate p.a. P.P.H. POCH Gliwice, polyvinyl alcohol 72000 (PVAL) P.P.H. POCH Gliwice, Thiomersal BP 1998 – Caesar & Loretz GmbH, phenylmercuric borate Pharma Cosmetic s.c. Cracow, chlorhexidine diacetate monohydrate – Fluka Biochemika, benzalkonium chloride NF, β-phenylethyl alcohol (2-phenylethanol) Merck-Schuchard.

Apparatus

pH-meter (CyberScan 500, Singapore); osmometer (Trident 800cl, Warsaw); Höppler viscosimeter KF10 (Prüfgeräte-Werk Medingen-Dresden); apparatus for membrane filtration – Sartorius; air sterilizer type S.P.W. 65M (Spółdzielnia Pracy Marki); autoclave EIMI type ESS-105 (Spółdzielnia Pracy Mechaników, Warsaw); densitometer (Mettler Toledo DA-110M); electronic analytical scales: up to 0.1 mg – type WPS 36/S and up to 0.002 g type WPS 720/C (Radwag, Radom).

Methods

Preparation of sterile solutions of additives

Citrate buffers I and II, solution of polyvinyl alcohol (PVAL), 0.5% solution of benzalkonium chloride, 2% solution of thiomersal, 0.04% solution of phenylmercuric borate and 1% solution of chlorhexidine diacetate. The solutions of additives mentioned above were prepared and examined using methods described in other publications (10, 11).

Preparation and studies of physicochemical stability of sterile buffered solutions of hydroxyethylcellulose (HEC), hydroxypropylmethylcellulose (HPMC) and polyvinyl alcohol (PVAL).

50.0 g of the solutions containing 0.7 g of HEC, 1.0 g of HPMC and 4.0 g of PVAL were prepared in separate flasks. The weight of the solution, after the filtration through a fritted glass funnel Schott G-1, was supplemented up to 100.0 g with

Table 1. Formulary composition of the eye drops containing cefazolin.

Constituents (per 100 g of the eye drops)	Formulary versions							
	I	II		III	IV		V	VI
		1	2		1	2		
Cefazolin	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Citrate buffer I Constituents of the buffer: Sodium citrate 3.0 Citric acid 0.15 Water 96.85	99.0	99.0	99.0	-	-	-	99.0	-
Citrate buffer II Constituents of the buffer: Sodium citrate 6.0 Citric acid 0.30 Water 93.70	-	-	-	49.5	49.5	49.5	-	49.5
Solution of polyvinyl alcohol (PVAL) viscosity η = 17.11 mPa·s	-	-	-	49.5	49.5	49.5	-	49.5
Thiomersal	-	0.002	0.02	-	0.002	0.02	-	-
Phenylmercuric borate	-	-	-	-	-	-	0.001	0.001
β-Phenylethyl alcohol	-	0.4	0.4	-	0.4	0.4	0.4	0.4

the preserved solution of citrate buffer, which was filtered through Schott G-3 and contained sodium citrate (6%), citric acid (0.3%), phenylmercuric borate (0.002%) and β-phenylethyl alcohol (0.5%).

The solutions were poured into infusion bottles and sterilized at the temperature of 120°C ± 2°C for 20 min. The freshly prepared solutions of hydrogels and those stored for 12 months at the temperature of 20°C were submitted to physicochemical evaluation, during which the following parameters were determined: color and clarity, pH, osmotic pressure and viscosity. The solutions of selected hydrogels, both freshly prepared and stored for 12 months, were colorless and clear.

HEC solutions.

Freshly prepared solution: pH 6.10 ± 0.1, osmotic pressure 369.00 ± 2.10, viscosity 29.24 ± 0.4 mPa·s. After 12 months of storage: pH 6.11±0.1, osmotic pressure 371.12 ± 3.20, viscosity 23.09 ± 0.3 mPa·s, viscosity loss 21.04%.

HPMC solutions.

Freshly prepared solution: pH 6.25 ± 0.2, osmotic pressure 372.20 ± 4.40, viscosity 11.21 ± 0.3 mPa·s. After 12 months of storage: pH 6.23 ± 0.2, osmotic pressure 368.00 ± 2.50, viscosity 10.18 ± 0.2 mPa·s, viscosity loss 9.19%.

PVAL solutions.

Freshly prepared solution: pH 6.10 ± 0.02, osmotic pressure 379.00 ± 1.73, viscosity 8.80 ± 0.18 mPa·s. After 12 months of storage: pH 6.14 ± 0.01, osmotic pressure 376.17 ± 2.40, viscosity 8.69 ± 0.01 mPa·s, viscosity loss 1.25%.

Studies of pharmaceutical compatibility of cefazolin with selected additives used in the technology of the eye drops

Under sterile conditions, selected additives were added separately into 1% solutions of aqueous cefazolin at following concentrations: sodium choride (0.9%), sodium citrate (6.0%), citric acid (0.3%), HEC (0.25%), HPMC (0.5%), PVAL (2.0%), thiomersal (0.002%-0.02%), phenylmercuric borate (0.001%), benzalkonium chloride (0.005%), chlorhexidine diacetate (0.01%), sodium pyrosulfite (0.01%), disodium EDTA (0.03%). Solutions of cefazolin containing the additives, protected from light, were stored at the temperature of 4°C and 20°C for 14 days and were examined for their color and clarity. The following examined additives were not compatible with 1% solution of cefazolin: Thiomersal at the concentration over 0.003%: sediment appeared after 6 days of storage at the temperature of 20°C and after 10 days at the temperature of 4°C.

Benzalkonium chloride at the concentration of 0.005% caused opalization immediately after mixing with 1% solution of cefazolin.

Chlorhexidine diacetate at the concentration of 0.01%, opalization appeared immediately after mixing with 1% solution of cefazolin, which was followed by precipitation during the storage at the temperature of 20°C and 4°C.

Preparation of the eye drops containing cefazolin

Under sterile conditions cefazolin was dissolved in the recommended volume of citrate buffer I or II (Tab. 1). After preservation, the solution was filtered through membrane filter with pore diameter 0.22 μm (Sartorius). The viscosity of the filtered eye drops was enhanced with the solution of PVAL. For the preservation of the eye drops 2% solution of thiomersal or 0.04% solution of phenylmercuric borate were used. The eye drops were stored at the temperature of 4°C and 20°C and protected from light. Qualitative assessment of freshly prepared eye drops and during their storage is presented in Tables 3-9.

Physicochemical evaluation of the eye drops containing cefazolin after their preparation and storage

Organoleptic analysis

The appearance of the drops, i.e. clarity, color

Table 2. Color, clarity and smell of the eye drops containing cefazolin after their preparation and during the storage at the temperature of 4°C and 20°C.

<table>
<tr><th rowspan="2">Formulary version of the eye drops</th><th rowspan="2">Freshly prepared eye drops</th><th colspan="2">10th day</th><th colspan="2">20th day</th><th colspan="2">30th day</th></tr>
<tr><th>4°C</th><th>20°C</th><th>4°C</th><th>20°C</th><th>4°C</th><th>20°C</th></tr>
<tr><td rowspan="3">I, II.1, III, IV.1, V, VI</td><td rowspan="4">Transparent, clear, smelling of antibiotic</td><td colspan="3">Colorless drops</td><td>Slightly yellow</td><td>Colorless</td><td>Slightly yellow</td></tr>
<tr><td colspan="3">No change of smell</td><td>More intense smell</td><td>No change of smell</td><td>More smell</td></tr>
<tr><td colspan="6">Clear</td></tr>
<tr><td>II.2, IV.2</td><td colspan="6">After 6 days of storage at the temperature of 20°C and after 10 days at the temperature of 4°C sediment appeared in the drops</td></tr>
</table>

Table 3. pH, osmotic pressure and viscosity of the eye drops containing cefazolin, freshly prepared and stored at the temperature of 4°C and 20°C.

Formulary version of the drops		pH			Osmotic pressure (mOsm/L)			Viscosity (mPa·s) of the drops after their preparation and after 30 days of storage (4°C and 20°C)
		Freshly prepared drops	30th day		Freshly prepared drops	30th day		
			4°C	20°C		4°C	20°C	
I		6.17 ± 0.01	6.11 ± 0.01	6.16 ± 0.01	342.33 ± 0.58	310.67 ± 3.78	325.33 ± 2.89	1.09 ± 0.01
II	1	6.18 ± 0.01	6.16 ± 0.01	6.18 ± 0.01	380.67 ± 2.08	347.67 ± 1.53	352.67 ± 2.39	1.09 ± 0.01
	2 (interaction)	6.16 ± 0.01	6.17 ± 0.02	6.17 ± 0.01	382.67 ± 3.11	360.11 ± 1.28	353.87 ± 4.13	1.09 ± 0.01
III		6.15 ± 0.02	6.14 ± 0.02	6.17 ± 0.01	368.33 ± 2.52	354.00 ± 2.64	350.33 ± 1.53	7.80 ± 0.13
IV	1	6.19 ± 0.02	6.20 ± 0.01	6.20 ± 0.01	384.67 ± 3.21	345.33 ± 3.06	342.33 ± 4.16	7.81 ± 0.14
	2 (interaction)	6.17 ± 0.01	6.16 ± 0.01	6.18 ± 0.01	387.22 ± 3.91	353.16 ± 4.01	348.20 ± 3.11	7.81 ± 0.13
V		6.15 ± 0.02	6.16 ± 0.01	6.20 ± 0.01	380.00 ± 3.00	343.67 ± 2.52	350.67 ± 3.21	1.09 ± 0.01
VI		6.16 ± 0.05	6.12 ± 0.02	6.21 ± 0.01	383.96 ± 3.54	352.67 ± 0.58	358.67 ± 2.52	7.88 ± 0.16

and smell, was assessed. The results of the analysis are presented in Table 2.

pH, osmotic pressure and viscosity of the eye drops

pH of the eye drops was determined with pH-meter, osmotic pressure was assessed with osmometer, while viscosity measurements were performed at the temperature of 20°C with Höppler viscosimeter. The results are shown in Table 3.

Density of the eye drops

Density of the eye drops was measured with densitometer. Density of the freshly prepared drops and of those stored at the temperature of 4°C and 20°C was in the range d_{20} = 1.0218 – 1.0275 g/mL.

Statistical analysis of the results of the studies of pH, osmotic pressure and viscosity was based on the formulary version **VI** (n = 4, f = 3, α = 0.05, $t_{\alpha f}$ = 3.182).
pH: $\bar{x}$ = 6.16, s = 0.03095, $S_{\bar{x}}$ = 0.01547, μ = 6.16±0.049, C_V = 0.5018%
Osmotic pressure: $\bar{x}$ = 383.96, s = 2.22669, $S_{\bar{x}}$ = 1.11346, μ = 383.96±3.54, C_V = 0.5799%
Viscosity: $\bar{x}$ = 7.88, s = 0.10488, $S_{\bar{x}}$ = 0.05244, μ = 7.88±0.16, C_v = 1.3309%.

Microbiological assessment of the eye drops containing cefazolin
Sterility studies

Sterility of the eye drops was confirmed with the method cited in PPh V using membrane filters. After 14 days of incubation on the liquid thioglikolane medium (PB1) and on the medium containing casein and soya hydrolysates (PB2), the bacterial growth was not observed.

Determination of antimicrobial activity (stability) of cefazolin in the eye drops using microbiological method

The antimicrobial activity of cefazolin in the freshly prepared drops and during their storage at the temperature of 4°C and 20°C was determined using cylinder-plate method, which is described in PPh V. The test strain *Staphylococcus aureus* ATCC 6538P was used. The results of the antimicrobial activity of cefazolin in the eye drops are shown in Table 4.
Statistical analysis of the precision of the method of the antimicrobial activity determination of cefazolin in the drops, in comparison to the standard solution, was based on the freshly prepared drops of formulary version **VI** (n = 9, f = 8, α = 0.05, $t_{\alpha f}$ = 2.306).
The antimicrobial activity of cefazolin in the standard solution: $\bar{x}$ = 27.61 mm, s = 0.05596, $S_{\bar{x}}$ = 0.01865, μ = 27.61 ± 0.04, C_V = 0.20%.
The antimicrobial activity of cefazolin in the drops:

Table 4. Results of the studies of the anti-microbial activity of cefazolin in freshly prepared drops and in those stored at the temperature of 4°C and 20°C in comparison with the standard substance (%).

Formulary versions of the drops		Anti-microbial activity of cefazolin in the eye drops in comparison with the standard substance (%) Test strain *Staphylococcus aureus* ATCC 6538P		
		Freshly prepared drops	**After 30 days of storage**	
			4°C	**20°C**
I		100.36 ± 0.73	100.00 ± 0.73	99.63 ± 0.36
II	1	100.00 ± 0.36	100.00 ± 0.36	100.00 ± 0.18
	2 (interaction)	100.00 ±1.55	100.00 ± 0.36	100.00 ± 0.18
III		99.92 ± 0.72	99.64 ± 0.36	100.00 ± 0.18
IV		1 99.27 ± 0.37	100.00 ± 0.36	100.00 ± 0.18
	2 (interaction)	99.27± 0.37	100.00 ± 0.18	99.63 ± 0.36
V		100.00 ± 0.18	100.00 ± 0.36	100.00 ± 0.33
VI		99.96 ± 0.20	100.00 ± 0.18	100.00 ± 0.18

$\bar{x}$ = 27.60 mm, s = 0.07070, $S_{\bar{x}}$ = 0.02357, μ = 27.60 ± 0.05, C_v = 0.2561%.

The antimicrobial activity of cefazolin in the drops is equivalent to 99.96% activity of cefazolin in the standard solution.

Studies of anti-microbial efficiency of preservatives: thiomersal and phenylmercuric borate in the eye drops containing cefazolin (preservation assay)

The antimicrobial efficiency of preservatives such as 0.002% and 0.02% thiomersal and 0.001% phenylmercuric borate mixed with 0.4% β-phenylethyl alcohol was examined with the preservation assay according to PPh V using test microbial strains mentioned in PPh V, i.e. *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC 6538, *Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 16404. Moreover, the bacteria which are dangerous for the eye but were not mentioned in the preservation assay of PPh V, were also included in the study. These were *MRSA* KO1, *Listeria monocytogenes* and *Bacillus cereus* KO2. Additional non-pharmacopeal test microorganisms, which were included in the studies of antimicrobial efficiency of preservatives in the drops, were clinical strains isolated from the eyes of patients. The results of the studies are presented in Tables 5 and 6.

Pharmaceutical interaction of thiomersal with cefazolin in the eye drops and studies of its influence on the physicochemical and microbiological properties of the eye drops

In order to find out the reason for the pharmaceutical interaction in the drops of versions **II.2** and **IV.2** (Tab. 1) the drops of formulary composition shown in Table 7 were prepared. The drops were clear immediately after their preparation. However, during their storage sediment appeared in the drops of formulary compositions: d, e and f, which proved that cefazolin interacted pharmaceutically with thiomersal, while the other constituents of the drops were compatible with cefazolin.

The consequences of the observed interaction were studied in the drops prepared according to the formulary composition presented in Table 7 (symbol d). In particular, it was examined whether the antimicrobial activity of cefazolin decreased and if thiomersal retained its required preservation efficiency in the eye drops. The studies were based on the separation of sediment on membrane filters of pores' diameter 0.22 μm and the determination of the antimicrobial activity of cefazolin in the drops (filtrates), followed by the preservation assay. The results are shown in Table 8 and 9.

The sediment collected on the filters was put into Kjeldahl flask, nitric acid of the concentration of 904 g/L was added and the mixture was heated for 6 min with a burner while being mildly boiled. After cooling it down and adding the solution of sodium hydroxide of the concentration of 398 g/L, yellow sediment appeared which might have been mercury oxide as the described analysis is cited in PPh V as the identification reaction for thiomersal.

RESULTS AND DISCUSSION

The eye drops submitted to analysis were 1% sterile aqueous solutions of cefazolin in citrate buffer of pH 6.15-6.20, preserved with 0.002%

Table 5. Degree of cell reduction of the test strains *Staphylococcus aureus ATCC 6538*. *MRSA*. *Pseudomonas aeruginosa ATCC 9027* in the eye drops containing cefazolin (preservation assay).

Eye drops containing 1% cefazolin				Degree of active cell reduction (%) after time (t)											
				Staphylococcus aureus ATCC 6538				***MRSA***				***Pseudomonas aeruginosa ATCC 9027***			
Version no.		Preservatives in the drops	concen-tration (%)	CFU/mL	6 h 99.00%*	24 h 99.90%*	28 days 100.00%*	CFU/mL	6 h 99.00%*	24 h 99.90%*	28 days 100.00%*	CFU/mL	6 h 99.00%*	24 h 99.90%*	28 days 100.00%*
II	1	thiomersal β-phenylethyl alcohol	0.002 0.4	8.9 x 10^5	99.94	99.90	100.00	8.9 x 10^5	99.82	99.82	100.00	8.4 x 10^5	99.71	99.94	99.99
	2	thiomersal β-phenylethyl alcohol (interaction)	0.02 0.4	6.8 x 10^5	99.95	100.00	100.00	7.7 x 10^5	99.88	100.00	100.00	7.0 x 10^5	100.00	100.00	100.00
IV	1	thiomersal β-phenylethyl alcohol (drops of increased viscosity)	0.002 0.4	8.1 x 10^5	99.98	99.99	100.00	8.6 x 10^5	99.93	99.93	100.00	8.4 x 10^5	99.62	99.80	99.80
	2	thiomersal β-phenylethyl alcohol (drops of increased viscosity) (interaction) 0.02	0.4	7.2 x 10^5	100.00	100.00	100.00	7.4 x 10^5	99.95	100.00	100.00	7.2 x 10^5	100.00	100.00	100.00
V		phenylmercuric borate β-phenylethyl alcohol	0.001 0.4	7.5 x 10^5	99.99	99.99	100.00	8.8 x 10^5	99.56	99.88	100.00	7.8 x 10^5	100.00	100.00	100.00
VI		phenylmercuric borate β-phenylethyl alcohol (drops of increased viscosity)	0.001 0.4	7.7 x 10^5	99.96	99.97	100.00	8.5 x 10^5	99.45	99.62	100.00	8.1 x 10^5	100.00	100.00	100.00

*) requiremants according to PPh V

Table 6. Degree of cell reduction of the test strains *Bacillus cereus. Listeria monocytogenes. Candida albicans ATCC 10231. Aspergillus niger ATCC 16404* in the eye drops containing cefazolin (preservation assay).

Eye drops containing 1% cefazolin				Degree of active cell reduction (%) after time (t)													
				Bacillus cereus				***Listeria monocytogenes***				***Candida albicans ATCC 10231***			***Aspergillus niger ATCC 16404***		
Version no.		Preservatives in the drops	Concen-tration (%)	CFU/mL	6 h 99.00%*	24 h 99.90%*	28 days 100.00%*	CFU/mL	6 h 99.00%*	24 h 99.90%*	28 days 100.00%*	CFU/mL	7 days 99.00%*	28 days**	CFU/mL	7 days 99.00%*	28 days**
II	1	thiomersal β-phenylethyl alcohol	0.002 0.4	7.7 x 10^5	99.95	99.92	99.95	7.1 x 10^5	99.99	99.99	100.00	7.5 x 10^5	99.97	99.99	7.0 x 10^5	99.99	100.00
	2	thiomersal β-phenylethyl alcohol (interaction)	0.02 0.4	7.0 x 10^5	99.96	99.90	99.94	7.1 x 10^5	100.00	100.00	100.00	6.9 x 10^5	100.00	100.00	6.9 x 10^5	100.00	100.00
IV	1	thiomersal β-phenylethyl alcohol (drops of increased viscosity)	0.002 0.4	7.5 x 10^5	99.90	99.89	99.90	7.5 x 10^5	99.99	99.99	100.00	7.5 x 10^5	99.99	100.00	7.1 x 10^5	99.99	100.00
	2	thiomersal β-phenylethyl alcohol (drops of increased viscosity) (interaction)	0.02 0.4	7.2x 10^5	99.90	99.88	99.80	7.1 x 10^5	100.00	100.00	100.00	6.2 x 10^5	100.00	100.00	6.5 x 10^5	100.00	100.00
V		phenylmercuric borate β-phenylethyl alcohol	0.001 0.4	7.8 x 10^5	99.89	99.85	99.89	8.0 x 10^5	100.00	100.00	100.00	6.0 x 10^5	100.00	100.00	6.4 x 10^5	100.00	100.00
VI		phenylmercuric borate β-phenylethyl alcohol (drops of increased viscosity)	0.001 0.4	7.5 x 10^5	99.88	99.84	99.89	8.5 x 10^5	100.00	100.00	100.00	6.4 x 10^5	100.00	100.00	6.8 x 10^5	100.00	100.00

*) requirements according to PPh V**) according to PPh V: after 28 days the volume of microorganisms is not expected to grow

Table 7. Pharmaceutical interaction of cefazolin in the eye drops.

Constituents of the drops	Volume (g) per 100 g of the drops	Symbols of the drops					
		a	b	c	d	e	f
Kefzol ®							
(Cefazolinum natricum)	1.0	+	+	+	+	+	+
Citrate buffer I	99.0	+	-	-	+	-	-
Citrate buffer II	49.5	-	-	+	-	-	+
solution of PVAL	49.5	-	-	+	-	-	+
(η=17.11 mPa·s)	99.0	-	+	-	-	+	-
thiomersal	0.02	-	-	-	+	+	+
interaction ↓		Clear solutions			↓ sediment *		

* particles of sediment appeared after 6 days of storing the drops at the temperature of 20°C and after 10 days at the temperature of 4°C

Table 8. Results of the studies of the antimicrobial activity of cefazolin in the eye drops containing sediment and after the separation of sediment.

Constituents of the drops	(g)	Antimicrobial activity of cefazolin determined using the test strain *Staphylococcus aureus ATCC 6538P* in comparison with the standard substance (%)		
		Freshly prepared drops	*After 30 days of storage at the temperature of 20°C*	
			Containing sediment	After the separation of sediment
Cefazolin Citrate buffer I Thiomersal	1.00 99.0 0 0.02	100.00 ± 1.41	100.00 ± 1.55	100.00 ± 1.85

thiomersal or 0.001% phenylmercuric borate mixed with β-phenylethyl alcohol (Tab. 1). PVAL proved to be the most useful for increasing the viscosity of the drops. After 12 months of storage at the temperature of 20°C the viscosity loss of PVAL solution was insignificant and equaled 1.25%, while under the same conditions the viscosity of HPMC solution decreased by 9.19% and the viscosity of HEC solution was lower by as much as 21.04%. Among examined additives, the pharmaceutical interaction with 1% solution of cefazolin was initiated by preservatives such as higher than 0.003% concentration of thiomersal, 0.005% benzalkonium chloride and 0.01% chlorhexidine diacetate.

The influence of the constituents of the drops on their physicochemical and microbiological stability was examined based on the analysis of the drops prepared according to six formulary versions (Tab. 1).

The drops, protected from light, were stored for 30 days at the temperature of 4°C and 20°C.

As the criteria of the qualitative assessment of the drops the following studies were considered: organoleptic analysis, pH, osmotic pressure, density, viscosity, antimicrobial activity (stability) of cefazolin in the drops and the preservation efficiency of thiomersal and phenylmercuric borate mixed with β-phenylethyl alcohol in the eye drops. The studies were also carried out in order to find out the reasons and consequences of the pharmaceutical interactions in the drops containing 0.02% thiomersal (Tab. 7, 8, 9).

During 30 days of storage the drops were clear with the exception of the drops of versions **II.2.** and **IV.2.**, in which sediment appeared (Tab. 2). The drops stored at the temperature of 4°C were clear, while those after 20 days of storage at the temperature of 20°C turned slightly yellow and had more intense smell. pH of the drops stored at both temperatures did not change, whereas the osmotic pressure of the drops decreased significantly (Tab. 3), although it stayed within the range of tolerance for the eye till the end of the studies.

The antimicrobial activity of cefazolin in the drops after 30 days of storage at both temperatures did not lower and retained 100% of its initial activi-

Table 9. Degree of cell reduction of the test strains in the eye drops containing sediment and after the separation of sediment (preservation assay).

Constituents of the drops	(g)	Test strain	Initial volume of cells CFU/mL	Degree of active cell reduction (%)					
				According to PPh V after 6 h 99.0%		According to PPh V after 24 h 99.9%		According to PPh V after 28 days 100.0%	
				1	2	1	2	1	2
cefazolin	1.0	*Staphylococcus aureus ATCC 6538*	8.7 x 10^5	99.95	98.72	100.00	99.99	100.00	100.00
		Pseudomonas aeruginosa ATCC 9027	9.1 x 10^5	100.00	99.97	100.00	99.99	100.00	100.00
		MRSA	8.2 x 10^5	99.88	99.89	100.00	99.95	100.00	100.00
citrate buffer I	99.0	*Bacillus cereus*	8.1 x 10^5	99.96	99.67	99.90	99.62	99.94	99.80
thiomersal	0.02	*Listeria monocytogenes*	7.9 x 10^5	100.00	99.89	100.00	99.92	100.00	100.00
						According to PPh V after 7 days 99.0%		According to PPh V after 28 days the volume of microorganisms is not expected to grow	
		Candida albicans ATCC 10231	8.5 x 10^5	-		100.00	99.68	100.00	100.00
		Aspergillus niger ATCC 16404	8.8 x 10^5	-		100.00	99.99	100.00	100.00

1 – drops containing sediment
2 – drops after the separation of sediment

ty. It was also true for the drops with the sediment, which, as the studies showed, contained thiomersal. The analysis proved that the storage temperature and used additives did not decrease the antimicrobial activity of cefazolin in the drops, which turned out to be much more stable than those of ceftazidime (10) and cefuroxime (11).

Used additives did not influence the results of the determination of the antimicrobial activity in the drops either. It is confirmed by the antimicrobial activity of cefazolin in the freshly prepared drops, which is very similar to the standard substance. After 30 days of storage at both temperatures, the viscosity of the drops, enhanced with polyvinyl alcohol up to 7.88 mPa·s, did not change (Tab. 3).

The results of the preservation assay showed that 0.002% thiomersal mixed with β-phenylethyl alcohol did not meet the requirements of PPh V in relation to the cell reduction of *Pseudomonas aeruginosa* ATCC 9027 (Tab. 5). However, 0.02% concentration, in spite of the presence of sediment, met those requirements. After the separation of sediment, the cell reduction of the test strains was too low and did not satisfy the requirements of PPh V (Tab. 9).

At the used concentration, phenylmercuric borate mixed with β-phenylethyl alcohol met the requirements of the preservation assay in relation to the pharmacopeal test strains and to *Listeria monocytogenes*. Slightly worse results were observed in case of the cell reduction of MRSA (Tab. 5) and *Bacillus cereus* (Tab. 6). The composition of the drops of versions **I**, **III**, **V** and **VI**, their pH (6.15-6.20), protection from light and storage at the temperature lower than 20°C guarantee physicochemical and microbiological compatibility of the drops with the requirements of PPh V and satisfactory microbiological stability of cefazolin in the eye drops; therefore, the drops containing cefazolin could be prepared in pharmacies as formulary medications.

REFERENCES

1. Dzierżanowska D.: Cephalosporic antibiotics (Polish). Medycyna Praktyczna, Kraków (1999).
2. Lambert H.P., O'Grady F.W.: Antybiotyki i chemioterapia. Wydawnictwo Medyczne, Warszawa (1994) – translation from English edited by Prof. J. Jeliaszewicz.
3. Sridhar M.S., Sharma S., Gopinathan U., Rao G.N.: Cornea 21, 718 (2002)
4. Bowe B.E., Snyder J.W., Eiferman R.A.: Am. J. Ophthalmol. 111, 686 (1991)
5. Khokhar S., Sindhu N., Mirdha B.R.: Infection 28, 149 (2000).
6. Bower K.S., Kowalski R.P., Gordon Y.J.: Am. J. Ophthalmol. 121, 712 (1996).
7. Charlton J.F., Dalla K.P., Kniska A.: Am. J. Health Syst. Pharm. 55, 463 (1998).
8. Ahmed I., Day P.: Am. J. Hosp. Pharm. 44, 2287 (1987).
9. How T.H., Loo W.Y., Yow K.L., Lim L.Y., Chan E.W., Ho P.C., Chan S.Y.: J. Clin. Pharm. Ther. 23, 41 (1998).
10. Kodym A., Zawisza T., Napierała B., Kukuła H.: Acta Pol. Pharm.- Drug Res. submitted.
11. Kodym A., Zawisza T., Taberska J., Kukuła H.: Acta Pol. Pharm. – Drug Res. submitted.

Received: 12.12.2005