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Effect of the buffer system on stability of tetracaine hydrochloride 0.5% eyedrops

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Abstract

The effect of the buffer system on the stability of tetracaine hydrochloride in eyedrop formulation was evaluated. Eyedrop formulations containing tetracaine hydrochloride 0.5%(w/v) were prepared using different buffer systems (acetate, phosphate and borate buffer) under a constant pH of 5.4, and a buffer concentration of 0.06M. Long-term tests at 26 °C and accelerated stability tests at elevated temperature (45, 50, 60 °C) over a period of 168 days were carried out by following the macroscopic view, pH, sterility, content of tetracaine hydrochloride and detection of the degradation products. Also, values of the constant of degradation rate at different temperatures and $t_{90\%}$ were calculated. The phosphate and acetate buffers provided satisfactory stability of tetracaine hydrochloride eyedrops, while borate buffer was not sufficient to maintain the pH value of the solution.

Key words: stability, tetracaine hydrochloride, eyedrops, buffer

Introduction

The estimation of stability is an important segment of the process of development of pharmaceutical dosage forms. The choice of optimal formulation is supported by good stability performance. Also stability tests are required for defining the expiry period and storage conditions (1,2).

Tetracaine hydrochloride, as a local anaesthetic agent, is indicated in ophthalmology for diagnostic purposes as well as for surgery and post surgery treatment (3). Eyedrop formulations contain 0.25-1%(w/v) of tetracaine hydrochloride in aqueous media. It should be prepared with respect of adjusting pH.

The pH value is a critical formulation parameter not only because of the physiological tolerance of the eye (4), but also becouse of the pH-dependent stability of tetracaine hydrochloride (5,6). Due to its ester structure, tetracaine hydrochloride is stable at a lower pH (7). As an ester-type compound, tetracaine hydrochloride undergoes hydrolysis, which results in the formation of n-buthyldimethylaminobenzoic acid and dimethylaminoethanol (5,8,9).

Different buffer systems could be used for adjusting the pH value of aqueous solutions of tetracaine hydrochloride intended to be used as eyedrops in a range from 3.7 to 6.5 (5). Generally, the selection of buffer agent should be made with respect not only to adjusting and maintaining the pH of the solution, but also to the type and concentration of buffer agent. Since the hydrolytic degradation of the drug is acid and base catalysed, it is obvious that the buffer concentration should be kept as low as possible to diminish this catalytic effects (1,10). Therefore, in the development stage of the liquid pharmaceutical dosage form, it is necessary to evaluate the stability of the drug substance in dosage form in terms of the catalytic effect of the buffer system. For this purpose, the drug degradation rate should be followed as a function of the type of buffer system in the formulation, while the pH and ionic strength are kept constant.

The aim of this study was to determine whether the type of buffer system (phosphate, acetate, and borate) affects the stability of tetracaine hydrochloride in 0.5%(w/v) eyedrop formulation with pH 5.4 and a buffer concentration of 0.06M.

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Experimental

Eyedrop formulation

Eyedrops containing 0,5% (w/v) tetracaine hydrochloride were prepared using different buffer systems: phosphate, acetate or borate (pH 5,4; 0,06M). Sodium chloride was used for isotonisation. No microbial preservative was added. Solutions were sterilised by use of bacteriological filtration. Formulations are given in table 1.

Stability testing

Long-term testing at 26 ^{0}C (II climate zone) (2) and short-term, accelerated tests at three elevated temperatures 45, 50 and 60 ^{0}C were used. Macroscopic evaluation (Ph.Eur.3), pH (Ph.Eur.3), sterility (Ph.Eur.3) and drug content (USP XX) were followed at certain time intervals over a period of 168 days. The obtained data were statistically processed. One way analysis of variance and the Scheffe method were used for processing of pH data. Rate constants of hydrolytic degradation of tetracaine hydrochloride at each temperature, the influence of temperature on the rate constant, as well as the $t_{90\%}$ at 26 ^{0}C were estimated (1,2):

$$\ln c = -kt + \ln c_o$$

$$\ln k = \ln A - Ea/RT$$

$$t_{90\%} = 0.105/k$$

where

c_o – *initial drug concentration (t=0)*
c – *drug concentration at time t*
k – *rate constant of the reaction of degradation (time $^{-1}$)*
t – *time*
A – *constant*
Ea – *activation energy*
R – *gas constant*
T – *absolute temperature*
$t_{90\%}$ – *time required for degradation of 10% of drug.*

In order to detect the degradation products, a HPLC method in accordance to the tetracaine hydrochloride eyedrops monograph (USPXXIII) was applied.

Results and discussion

Stability studies on prepared eyedrop formulations of tetracaine hydrochloride 0.5% (w/v) indicated certain changes in the parameters followed.

The long-term study at 26 ^{0}C, did not show any macroscopic change in all three eyedrops formulations over a period of 168 days. Exposure to an elevated temperature (accelerated stability tests) caused very slight red-brownish colouring of the solutions (table 2). The coloration of formulation 1, containing phosphate buffer, was registered after 84 days of storage at 50 ^{0}C and 56 days at 60 ^{0}C, while in the presence of acetate buffer it was noticed after 56 days (45 ^{0}C), 28 days (50 ^{0}C) or 14 days (60 ^{0}C). Formulation 3, prepared using a borate buffer, showed slight coloration only at the highest temperature of 60 ^{0}C after 56 days. The red-brown colour is probably a result of decomposition of n-buthy-laminobenzoic acid, induced by extreme temperature (5,11).

The follow up of the pH value is an important parameter since the decomposition of tetracaine hydrochloride results in pH decrease. Table 3 shows the statistical parameters obtained from pH data by use of one way analysis of variance and the Scheffe method. A statistically significant change of pH value within a storage period of 168 days at 26 ^{0}C was registered only in the eyedrop solution containing borate buffer. Exposure to an elevated temperature facilitated the pH changes in all samples. During the accelerated stability tests, acetate buffer provided the most stable pH value. Taking into consideration the corresponding buffer capacities of the three buffer systems used (table 4), the 0.06M borate buffer has a very low buffer capacity (10^{-6} M HCl), while the same molarity of acetate or phosphate buffer provides higher buffer capacities (10^{-2} and 10^{-4} M HCl respectively). Therefore, an acetate buffer enables the most efficient maintaining of the pH value, while the buffer capacity of borate buffer was insufficient.

Table 1. Tetracaine hydrochloride eyedrop formulations

	formulation		
components	1	2	3
tetracaine hydrochloride	0.5 g	0.5 g	0.5 g
sodium chloride	0.471 g	0.479 g	0.617 g
phosphate buffer 0.06 M, pH 5.4	ad 100ml		
acetate buffer 0.06 M, pH 5.4		ad 100ml	
borate buffer 0.06 M pH 5.4			ad 100ml

Changes in the content of tetracaine HCl during the stability testing is presented in figure 1. Table 5 shows the rate constants of hydrolytic degradation of tetracaine hydrochloride at each temperature, as well as the $t_{90\%}$ at 26 ^{0}C.

As can be seen, the slowest rate of hydrolytic degradation at each temperature occurred in the sample containing borate buffer (table 5). The time required for degradation of 10% of drug substance ($t_{90\%}$) at 26^{0}C was 318 days. This could be explained by: (a) an insufficient buffer capacity of borate buffer (table 4) that allowed a decrease of pH value and (b), an increased stability of tetracaine hydrochloride at a lower pH on the other hand. The stability of tetracaine hydrochloride in formulations containing phosphate and acetate buffer is similar

Table 2. Macroscopic changes of samples 1-3 exposed to different temperatures

temperature	26°C	45 °C				50 °C				60 °C			
time (days)	0 - 168	0	28	56	84	0	28	56	84	0	14	28	56
sample 1	—	—	—	—	—	—	—	—	+	—	—	—	+
sample 2	—	—	—	+	+	—	+	+	+	—	+	+	+
sample 3	—	—	—	—	—	—	—	—	—	—	—	—	+

\+ macroscopic changes - no changes

Table 3. Statistical analysis of pH value data - one way analysis of variance and Scheffe method

26 °C						
sample	F_t	F	F-ratio for			F'
			x_0 and x_{28}	x_0 and x_{56}	x_0 and x_{84}	x_0 and x_{168}	
1		3.46	-	-	-	-	
2	3.48	3.37	-	-	-	-	13.92
3		113.82	9.64	17.14	213.34	289.74	
45 °C						
sample	F_t	F	F-ratio for			F'
			x_0 and x_{28}	x_0 and x_{56}	x_0 and x_{84}	
1		116.48	31.50	87.60	330.30	
2	4.07	5.19	5.13	10.05	13.13	12.21
3		173.05	217.56	342.25	425.39	
50 °C						
sample	F_t	F	F-ratio for			F'
			x_0 and x_{28}	x_0 and x_{56}	x_0 and x_{84}	
1		73.79	46.88	142.24	181.51	
2	4.07	18.47	8.96	26.74	50.07	12.21
3		209.93	244.45	412.58	522.16	
60 °C						
sample	F_t	F	F-ratio for			F'
			x_0 and x_{14}	x_0 and x_{28}	x_0 and x_{56}	
1		110.82	129.94	211.76	280.06	
2	4.07	22.58	11.88	32.06	62.06	12.21
3		287.90	330.67	599.76	689.05	

($t_{90\%}$ on 26°C was 181 and 157 days respectively). The degradation rate constant k versus temperature plots for all three formulations are shown comparatively in figure 2. A significant increase in the degradation rate by increasing the temperature is obvious. For phosphate and acetate buffer, by increasing the temperature, k values (day^{-1}) have been changed from 10^{-4} order (26 °C) to 10^{-2} (60 °C).

In order to register the products of degradation of tetracaine hydrochloride, HPLC studies were carried out. A decrease of the

Table 4. Calculated values of buffer capacities

buffer	$\beta_{for\ acid}$ (M HCl)
phosphate buffer (0.06M pH 5.4)	8.41×10^{-4}
acetate buffer (0.06M pH 5.4)	3.05×10^{-2}
borate buffer (0.06M pH 5.4)	7.74×10^{-6}

Table 5. Degradation rate constant (k) and $t_{90\%}$ of tetracaine hydrochloride in eyedrop formulations 1-3 at different temperatures

Sample	k(day^{-1})				$t_{90\%}$(days)			
	26 °C	45 °C	50 °C	60 °C	26 °C	45 °C	50 °C	60 °C
1	$5.80 \cdot 10^{-4}$	$3.31 \cdot 10^{-3}$	$4.63 \cdot 10^{-3}$	$1.51 \cdot 10^{-2}$	181	31.7	22.7	6.9
2	$6.70 \cdot 10^{-4}$	$4.60 \cdot 10^{-3}$	$6.84 \cdot 10^{-3}$	$2.04 \cdot 10^{-2}$	157	22.8	15.3	5.1
3	$3.30 \cdot 10^{-4}$	$1.18 \cdot 10^{-3}$	$1.98 \cdot 10^{-3}$	$4.07 \cdot 10^{-3}$	318	89.0	53.0	25.8

tetracaine hydrochloride quantity in aged samples was accompanied by the appearance of two new peaks in all three formulations. Figure 3 represents comparatively the HPLC chromatograms of sample 1 freshly prepared (a), stored for 56 days at 60 ^{0}C (b) and standard of PABA (c). Comparison of the chromatograms clearly indicates a decrease in the concentration of tetracaine hydrochloride (retention time Rt_1 4.33±0.03) during ageing and the appearance of two new peaks in aged samples (Rt_2 2.42±0.02; Rt_3 3.12±0.01), which could be related to the degradation products (5,9). Since the retention time of standard of PABA (2.41±0.01), proposed as a standard for the detection of related substances (BP99, Tetracaine hydrochloride eyedrops

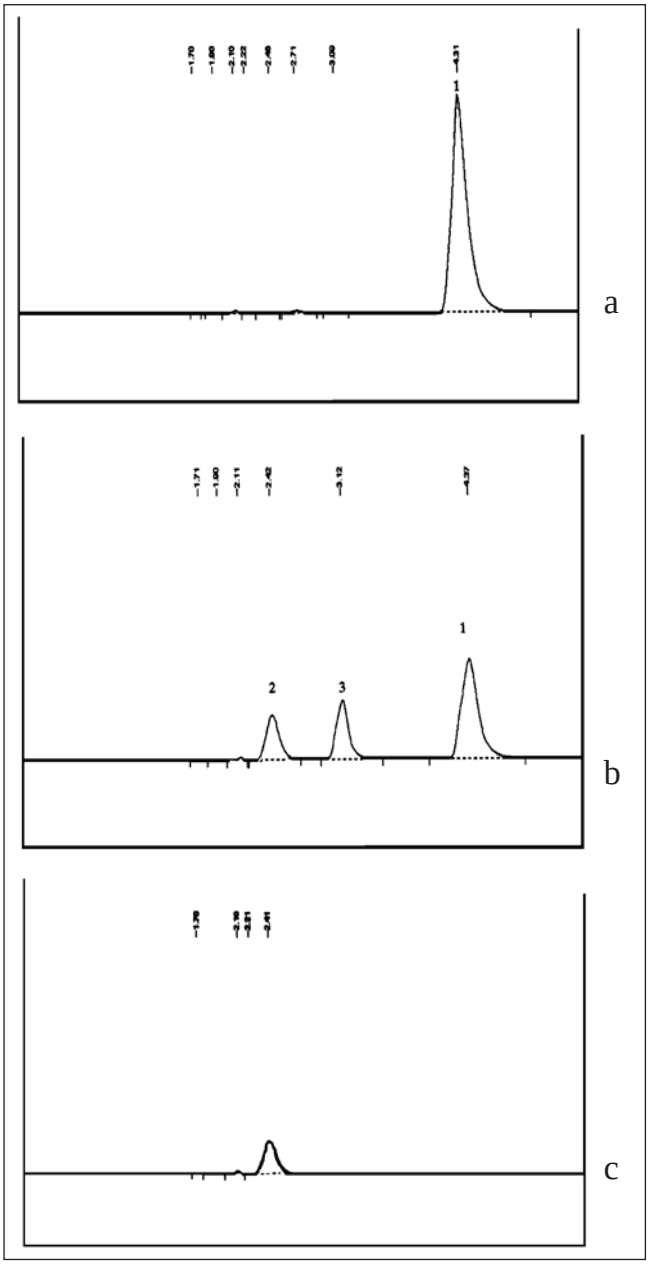

Fig 3. HPLC chromatograms of sample 1: freshly prepared (a), stored for 56 days at 60 ^{0}C (b)a nd standard of PABA (c).

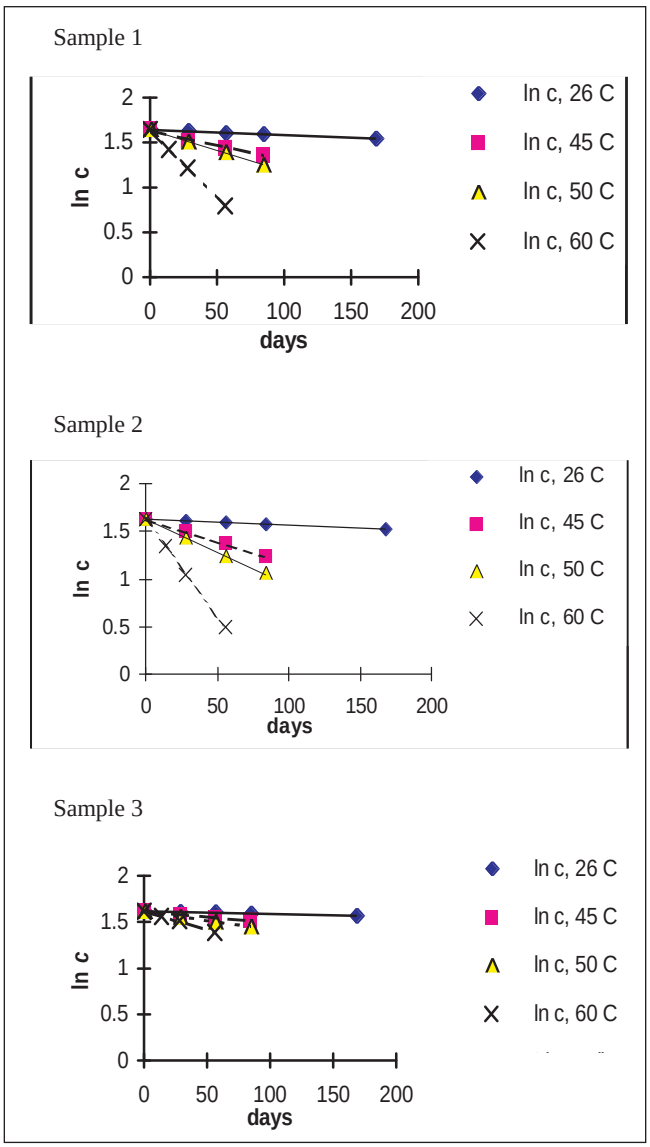

Fig 1. ln c (n =3) versus time plots of samples 1-3 at different temperatures

monograph), corresponds to Rt_2, this peak is related to the n-buthyldimethylaminobenzoic acid.

Regarding the sterility of eyedrop solutions, all three samples remain sterile within a stability testing period of 168 days.

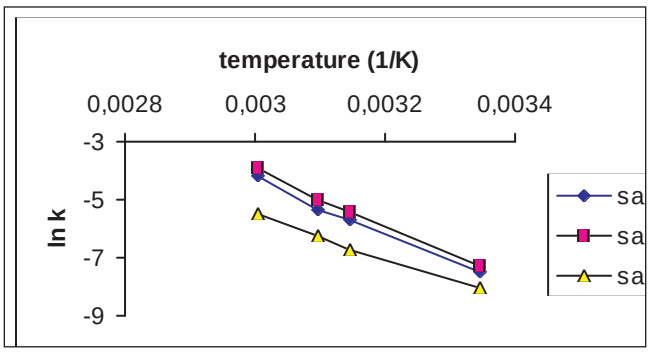

Fig 2. Arrhenius plot for three formulations of tetracaine hydrochloride 0,5% eyedrops

Conclusion

The stability of the tetracaine 0,5%(w/v) eyedrop solution with pH 5,4 and a constant buffer concentration of 0.06M is a function of the buffer system used in formulation. Incorporation of a phosphate or acetate buffer 0.06M with pH 5.4 in formulation of 0,5%(w/v) tetracaine hydrochloride eyedrops provided satisfactory stability ($t_{90\%}$ 181 and 157 days respectively). Although the degradation rate of tetracaine hydrochloride in the formulation containing 0,06M of borate buffer was twice as slow ($t_{90\%}$ 318 days), this formulation is not acceptable because its poor buffer capacity was insufficient to maintain the pH value.

References

1. J. Carstensen, *Drug Stability,* Marcel Dekker, New York,1990
2. W. Grimm, G.Schepky, *Stabilitatpufung in der Pharmazie, Theorie und Praxis,* Ed. Cantor Aulendorf, 1980.
3. *USP Drug Information for the Health Care Professionals,* vol.1, 16th ed., US Pharmacopeial Convention Inc 1997.
4. K.E. Avis, H.A.Lieberman and L.Lachman, Ed., *PharmaceuticaL Dosage Forms,* Parenteral Medications, Marcel Dekker, New York, 1992.
5. R. Dolder and F.Skinner, *Ophtalmica,* Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart 1990
6. D.B. Kersten and B.Gober,U.Tim,*Pharmazie* **36**,341(1981)
7. B. Gober, U.Timm and S.Pfeifer, *Pharmazie* **34** (3),161-164 (1979)
8. J. Breinlich, *Pharm..Ztg.***110**, 579 (1965)
9. G.N. Menon and B.J.Norris, J.*Pharm.Sci.,* **70**(5),569-570 (1981)
10. L. Lachman, H.A. Lieberman and J. Kanig, Ed.,*The Theory and Practice of Industrial Pharmacy,* Lea &Febiger, Philadelphia, 1970.
11. W. Lund, Ed., *The Pharmaceutical Codex,* 12th ed.,The Pharmaceutical Press, London, 1994.

Резиме

Ефект на пуферскиот систем врз стабилноста на капки за очи со 0,5% тетракаин хидрохлорид

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Клучни зборови: стабилност, тетракаин хидрохлорид, очни капки, пуфер

Испитуван е ефектот на пуферскиот систем врз стабилноста на тетракаин хидрохлоридот во очни капки. Приготвени се три формулации на очни капки со 0,5% тетракаин хидрохлорид во кои е употребен различен пуфер (фосфатен, ацетатен или боратен) во концентрација 0,06M и pH вредност 5,4. Стабилноста е испитувана со примена на долготрајни тестови (26 °C) и забрзани тестови за следење на стабилноста (45, 50, 60 °C) во тек на период од 168 дена. Следени се параметрите: макроскопски изглед, pH содржина на тетракаин хидрохлорид и деградациони продукти и стерилност. Определени се константите на брзина на разградба на тетракаин хидрохлоридот на различни температури, како и времето на разградба на 10% од активната компонента $t_{90\%}$. Фосфатниот и ацетатниот пуфер обезбедија задоволителна стабилност на очните капки со тетракаин хидрохлорид, додека боратниот пуфер поради нискиот пуферски капацитет не беше во можност да ја одржи pH вредноста на капките за очи.