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Systematic study of factors affecting eye drop size and dosing variability

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The application of eye drops is known to be problematic due to the high variability of eye drop volume, the low capacity of the precorneal area resulting in an optimal drop volume of about 20 μl, and the risk of adverse systemic effects of drugs due to systemic absorption via the nasal mucosa. While dropper tip design and the surface activity of the antimicrobial preservative strongly influence the volume of an aqueous ophthalmic solution dispensed as eye drops, the handling of the preparation (dispensing angle, dispensing rate, and the residual volume of liquid in the dropper bottle) is generally believed to produce a minimal effect. In this study, properties of two dropper tips (rubber and plastic) frequently used in the Czech Republic were systematically investigated in a fractional factorial experiment. Of seven determinants potentially influencing the size of eye drops, the dropper tip design, the dispensing angle and the dispensing rate have been found to have a significant effect on the eye drop volume. Wetting of the rubber dropper tip resulted in a dramatic increase in drop volume which could hardly be foreseen in real drop dispensing. As a result, therefore, rubber dropper tips could scarcely be recommended, as opposed to plastic dropper tips which produced drops of comparable volume when used in upright position with approximately the same dispensing rate. Under those defined dispensing conditions, the variability of the drop volume could be expressed by a variability coefficient of 3.3%. Using a dispensing angle of 45° from horizontal led to a decrease in drop volume and, in addition, to greater volume variability due to the formation of air bubbles inside the dropper tip chamber.

1. Introduction

An aqueous ophthalmic solution to be instilled drop-wise in the lower conjunctival sac of the patient remains the preferred dosage form for ocular medication since administration is relatively easy and well tolerated and causes minimal discomfort when properly formulated (Kaur and Kanwar 2002). The instillation of a normal, non-viscous aqueous solution in the eye results in a pulse-dose action profile, caused by the limited preocular residence time and the low bioavailability of the drug (Järvinen et al. 1995). The volume of one drop of a topically applied ophthalmic preparation strongly influences the therapeutic action of drug, but, may also affect the adverse systemic effects of the drug due to systemic absorption (Urtti and Salminen 1993). Optimally, a drop size smaller than 20 μl is preferred for ophthalmic drugs to improve the therapeutic effect (Ludwig and Van Ooteghem 1986) and to decrease the adverse effects. The drop size of commercially available ophthalmic solutions generally varies between 25 and 70 μl with an average value of about 40 μl (Lederer and Harold 1986).

The size of a drop delivered from a flexible dropper bottle is influenced by three groups of effects: the design and characteristics of the dropper tip and dropper bottle (Brown et al. 1985; Van Santvliet and Ludwig 1999a, 2001), the physico-chemical properties of the solution to be dispensed (Van Santvliet and Ludwig 1999d), and the patient's handling of the dropper bottle (Van Santvliet and Ludwig 1999b). The inner diameter and the width of the flat end of the dropper tip, the surface tension and viscosity of the solution, and the dispensing angle and dispensing rate have been found the most important.

In the first part of the study, to test the properties of two different Czech made dropper tips, seven main factors which could influence the drop delivery were examined at two different levels. In this case, a full experimental design requires 2^7, i.e. 128 experimental runs with a high number of multi-factor interactions which, of course, are assumed to be negligible for the volume of drops tested. A 2^{7-3}_{IV} fractional factorial design with resolution IV in which no main factor is confounded with any other main factor or two-factor interaction, but where two-factor interactions are confounded with two others, was used in our study. This aggregate design uses 2^4, i.e. sixteen experimental runs and makes it possible to estimate all the seven main factors clear of the two-factor interactions. The generated relations for all sixteen runs of the 2^{7-3}_{IV} fractionals use three independent generators G_1 1235, G_2 1246 and G_3 1347 (Box and Hunter 2000).

In the second part of the study, the influence of the concentration of a surface-active solute (benzalkonium chlor-

Table 1: Levels of factors

Code	Factor	Factor level	
		Low (−)	High (+)
A	Preservation (0.01%)	TIM	BAC
B	Dropper tip (material)	Plastic	Rubber
C	Dispensing angle (°)	45	90
D	Volume of liquid (ml)	3	10
E	Drops (number)	10	20
F	Bottle size (ml)	10	25
G	Drop delivery rate	Slow	Quick

ide) on the volume of drops delivered from the plastic bottle fitted with two different dropper tips using two different dispensing angles was investigated in detail.

2. Investigations and results

2.1. Screening of significant factors

The seven factors investigated are identified by capital letters. Their two levels (lower and higher) are illustrated in Table 1. The levels of the qualitative factors A, B, and G have been allotted a conventional character. The higher level of surface activity (factor A) corresponds to the use of a 0.01% solution of the surface-active preservative benzalkonium chloride (BAC), while a 0.01% solution of thimerosal (TIM) without surface activity represents the lower level of this factor. The higher level of factor B is attributed to a rubber dropper tip as opposed to the lower level, attributed to a plastic dropper tip. The slow delivery of individual drops is the low level of factor G as distinct from the rapid consecutive delivery of drops at the higher level of factor G. The levels of the quantitative factors C, D, E, and F correspond to the numerical values shown in Table 1.

The experimental design matrix comprising 16 experimental runs of the actual factor combinations shown as one of two alternatives at their higher (+) or lower (−) level, is summarised in the left-hand part of Table 2. The values in the of last two columns of Table 2 compare drop volumes (µl) measured in each of the 16 experiments with those estimated using the analysis of variance (ANOVA) summarised in Table 3.

Table 2: Actual experimental design matrix and results

Run number	A	B	C	D	E	F	G	Volume of drop (µl)	
								Measured	Estimated
1	−	−	−	−	−	−	−	39	41.00
2	+	−	−	−	+	+	+	49	48.75
3	−	+	−	−	+	+	−	44	44.75
4	+	+	−	−	−	−	+	46	45.50
5	−	−	+	−	+	−	+	42	44.50
6	+	−	+	−	−	+	−	48	45.25
7	−	+	+	−	−	+	+	53	48.75
8	+	+	+	−	+	−	−	58	56.50
9	−	−	−	+	−	+	+	49	45.00
10	+	−	−	+	+	−	−	37	37.25
11	−	+	−	+	+	−	+	48	49.25
12	+	+	−	+	−	+	−	48	48.50
13	−	−	+	+	+	+	−	45	49.00
14	+	−	+	+	−	−	+	50	48.25
15	−	+	+	+	−	−	−	49	52.75
16	+	+	+	+	+	+	+	45	45.00

Table 3: ANOVA statistical data ($p > 0.05$)

Factor	Sum of squares	Degree of freedom	Mean square	F-value	Critical $F_{0.05;\,1,10}$
B	64.00	1	64.00	7.1111	
C	56.25	1	56.25	6.2500	
AD-BF-CG	72.25	1	72.25	8.0278	4.9646
G	56.25	1	56.25	6.2500	
BG-CF-DE	49.00	1	49.00	5.4444	
Residual	90.00	10	9.00		
Sum	387.75	15			

In Table 3, only the significant ($p > 0.05$) individual factors and confounded groups of two-factor interactions are listed. Besides the non-confounded individual factors B, C, and G, the two-factor interactions BG and CG indicated by bold letters were significant. All the remaining factors and multi-factor interactions with complementary relevance were found to be non-significant.

2.2. Drop volume

In the next part of the study, the influence of two significant factors, first, B (dropper tip type, either plastic or rubber), and second, C (dispensing angle, 90° or 45° from horizontal, respectively), on eye drop volume was investigated in detail. The third significant factor G (the drop delivery rate) has only experimental relevance since, in practice slow delivery of one individual drop is required during eye drop dispensing.

There is one important circumstance associated with the use of the rubber dropper tip, the wetting of the dropper tip orifice which influences the functional cross-sectional diameter at which a drop is formed. In this article, non-wetted (NW) and wetted (W) rubber dropper tips are distinguished.

In Fig. 1 and Fig. 2, the average drop volumes (µl) of twenty individual drops ($n = 20$) of five solutions of BAC are shown with confidence levels (mean ± 1.96 SD) for probability $p = 0.05$. In the order plastic, non-wetted rubber (NW) and wetted (W) rubber dropper tips, Fig. 1 shows results obtained with a dispensing angle of 90° in contrast to Fig. 2 which shows results obtained at a dispensing angle of 45° from the horizontal.

At both the dispensing angles investigated, the wetted rubber dropper tip (W) produced significantly larger drop volumes ranging from 53.3 to 74.4 µl. No significant differences between the drop volumes obtained for plastic and

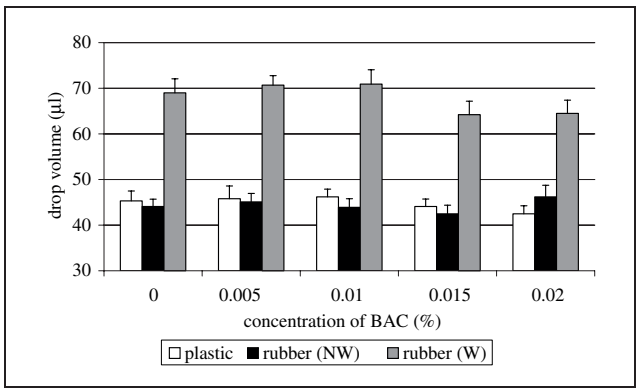

Fig. 1: Influence of dropper tip on average drop volume ($n = 20$) of 0%–0.005%–0.01%–0.015%–0.02% solutions of BAC at a dispensing angle of 90°

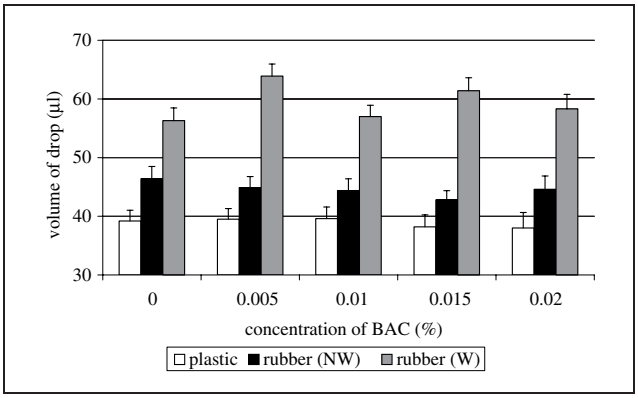

Fig. 2: Influence of dropper tip on average drop volume (n = 20) of 0%–0.005%–0.01%–0.015%–0.02% solutions of BAC at a dispensing angle of 45°

non-wetted rubber dropper tips were observed at a 90° dispensing angle. The drop volumes ranged from 41.3 to 48.1 µl. Decreasing the dispensing angle from 90° to 45° from the horizontal results in significantly smaller drop volumes in the case of plastic dropper tips, and, except for the 0.015% solution of BAC, for the wetted rubber dropper tip as well. Although the addition of various concentrations of the surface-active preservative BAC resulted in a small change in average drop volumes, even in this detailed experiment, the effect of the presence of BAC was ambiguous and not significant.

Besides the individual drop volume, however, variability of volume is also very important in the dispensing of eye drops. If the addition of various concentrations of BAC was found to produce no significant effect on drop volume in this study, the volumes of all one hundred individual drops of the five BAC concentrations (n = 100) could, therefore, be provided for the estimation of total average drop volume (µl). In Table 4, the drop delivery data: average drop volume (µl), standard deviation (SD) and confidence intervals (mean ± 1.96 SD, p = 0.05) are presented for plastic, non-wetted rubber and wetted rubber dropper tips, according to the dispensing angle used. To compare variability of drop volumes, relative standard deviations (RSDs) were used as described in the last column of Table 4. Estimated RSDs varied within the range of 3.3 to 5.2%. The largest drop volumes (53.3 to 74.4 µl) and RSDs (4.9 to 5.2%) were recorded for the wetted rubber dropper tip. Drop volumes in the range of 38.9–44.8 µl represent the results of the comparable drop delivery for plastic and non-wetted rubber dropper tips at both dispensing angles, although a significant decrease in drop volumes was found for the plastic dropper tip at a 45° angle. The very low RSDs observed (from 3.3 to 3.8%) support systematic training in drop delivery technique.

Table 4: Influence of dropper tip and dispensing angle on average drop volume (µl) and drop volume variability

Dispensing angle	Dropper tip	Average drop volume (µl)	SD	Intervals	RSD (%)
90°	Plastic	44.8	1.7	41.5–48.1	3.8
	Non-wetted rubber	44.4	1.6	41.3–47.5	3.6
	Wetted rubber	67.9	3.3	61.4–74.4	4.9
45°	Plastic	38.9	1.3	36.5–41.3	3.3
	Non-wetted rubber	44.6	1.5	41.6–47.6	3.4
	Wetted rubber	59.4	3.1	53.3–65.5	5.2

3. Discussion

The 2^{7-3}_{IV} fractional design is of value in screening experiments where a large number of factors might be important but where only few are dominant. In those cases, the assumption is made that a simple structure of individual significant factors and their two-factor interactions will be obtained and that higher-order interaction effects are negligible (Box and Hunter 2000). In Table 3, three main factors are shown to be significant: dropper tip type (factor B), dispensing angle (factor C), and drop delivery rate (factor G), as well as their two-factor interactions BG and CG that represent a group of three confounded two-factor interactions. By using the ANOVA data, estimation of regression coefficients allowed a regression equation (1) to be obtained, which enables 77% of the total sum of squares to be explained:

$$V[\mu l] = 46.875 + 2.000 \cdot B + 1.875 \cdot C - 1.875 \cdot G - 1.750 \cdot BG - 2.125 \cdot CG \quad (1)$$

The minus or plus sign of each regression coefficient indicates the negative or positive effect of the significant factor on eye drop volume. Eq. (1) was used to calculate the estimated volumes of drops for each of the 16 experimental runs given in Table 2. The successful use of Eq. (1) is restricted by taking +1 or −1 instead of the corresponding independent factors and their interactions. Our findings here are in good agreement with a literature review (Van Santvliet and Ludwig 1999a, 1999b, 1999c, 1999d, 2001). Surprisingly, in comparison with the results of other investigators, no significant effect of addition of the surface-active preservative benzalkonium chloride was found in our study. We supposed, firstly that this could possibly result from the only two-level experiment, and secondly, through the probable complex effect of surface tension on drop volume in interactions with the other factors. The influence of significant factors was investigated in detail in the next part of our study.

Ophthalmic solutions are presented in a wide variety of dropper bottles fitted with several types of dropper tips. One of the main additional factors that directly determine the volume of a dispensed drop is the external orifice width of the flat end of the dropper tip from which a drop falls (Brown et al. 1985). This is very important in the case of highly wetted dropper tip materials such as rubber, since the eye drop size increases linearly with the outer diameter. In Fig. 1 and Fig. 2, significant differences between the wetted (W) and the non-wetted (NW) rubber dropper tips are illustrated. If the surface at which a drop forms is highly wetted, the wetted rubber dropper tip produces the largest eye drops, or, if it is defined less precisely through poorly controlled wetting during repeated administration of drops, the volume of drops increases gradually. This could be seen frequently during the experiments simply by eye, and, in those cases, in fact, some experimental series had to be eliminated. The observed differences in drop size might not have a major impact when the solution is used for the treatment of dry eyes, but they could be very problematic in the case of highly-active substances where an accurate dose of a drug needs to be administered. The differences in wetting of the rubber could arise not only from repeated contact of the liquid inside with the rubber tip when a drop is formed and expelled, but also with changes in the properties of the rubber material over time. As a result, wetted rubber dropper tip can scarcely be recommended for practical drop delivery. At present, in the Czech Republic,

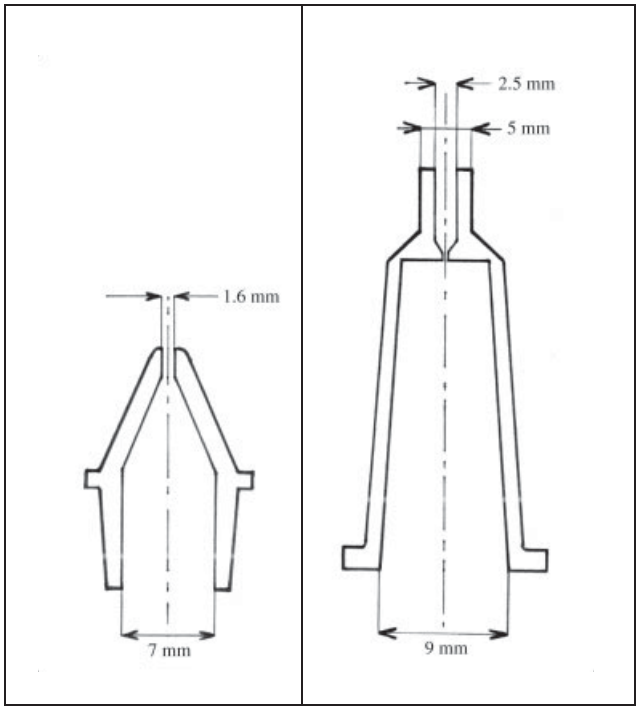

Fig. 3: Dropper tips investigated

rubber dropper tips are usually used for ophthalmic preparations without drugs, of which artificial tears are a typical example.

Nowadays, ophthalmic solutions are mostly presented in flexible plastic containers fitted with plastic dropper tips (Van Santvliet et al. 1996, 2004). In general, no perceptible trends toward changes of drop volume due to wetting were observed during a systematic study of drop dispensing. On the other hand, air bubble formation inside the tip capillary was often noted. This usually resulted in obstructed passage of liquid resulting in increased variability of individual drop volumes that, however, did not exceed the generally tolerated range of 20% (Van Santvliet and Ludwig 1999a). Dispensing drops from a plastic dropper tip at an angle of 45° from the horizontal significantly decreased the average drop volume as illustrated in Fig. 2 and Table 4. As a result of gravity, tilting from a vertical position to 45° from the horizontal reduces the perimeter of the outer orifice of the dropper tip, at which a drop is formed, and smaller drops would be expected (Van Santvliet and Ludwig 2001).

In so far as the design and dimensions of the dropper tip and the physico-chemical properties of the dispensed solution are selected by the manufacturer, eye drop volume can be influenced strongly only by the patient's manipulation technique, in particular by the dispensing angle, drop dispensing rate, or repeated dosing, respectively (Van Santvliet and Ludwig 2004). No guidelines concerning the drop dispensing system, the drop volume of the dispensed solution or the convenience for the patient of eye drop medication bottles are available. Generally, to form a drop, the bottle has to be inverted and squeezed carefully. In our experiments, when the bottle was inverted too quickly, one or more drops fell spontaneously. In this case there is no possibility of controlling their volume. It is known that a reduced drop formation rate leads to a decrease in drop volume in contrast to a higher drop formation rate when the tail of liquid created by the falling drop results in an extra pulse of liquid into the drop and hence an increase in drop volume (Van Santvliet and Ludwig 1999d, 2001). For this reason, if drop delivery follows in a regular manner, slow squeezing of the plastic dropper bottle results in a slow drop formation rate allowing the individual drop volume and hence drop volume variability to be controlled, as demonstrated in Table 4. As a result of our study, wherever an accurate dose of the drug needs to be administered, a plastic dropper tip may be recommended and the patients should be instructed on the correct technique of slow administration of individual drops at a dispensing angle of 90°.

4. Experimental

4.1. Materials

Two different dropper tips made of low-density polyethylene were obtained from two Czech manufacturers. The plastic dropper tip differed from the rubber dropper tip in the diameter and design of the outer orifice. In the schematic, cross-sectional drawing (Fig. 3), it can be seen that while the plastic dropper tip (left) had a hemispherical surface surrounding the outer orifice, the rubber dropper tip (right) had a straight end.

The dropper tips were fitted to the different round plastic dropper bottles (10 ml or 25 ml, respectively) also made of low-density polyethylene.

4.2. Preparation of solutions

Two different antimicrobial preservatives, both of pharmaceutical grades, were examined: thimerosal (TIM) (Acros Organics, New Jersey, USA) and benzalkonium chloride (BAC) (Acros Organics, New Jersey, USA). All solutions were prepared by adding the required amount (TIM) or volume (BAC), respectively, of preservative to water to achieve the required concentration (% w/v or % v/v, respectively), and were kept at room temperature. Deionised freshly double-distilled water was used throughout the study.

4.3. Drop dispensing

Depending on the individual experimental run in the screening experiment, 3.0 or 10.0 ml of the 0.01% preservative solution was filled into plastic dropper bottles of 10 or 25 ml, respectively, fitted with a plastic or rubber dropper tip, respectively. The bottle was squeezed in the upright position (90° angle) or at 45° from the horizontal until the required number of drops was obtained. In the slow administration rate experiment, 10 or 20 drops, respectively, are administered drop by drop by slow careful squeezing of the dropper bottle with a time interval of 1 drop per second into a glass beaker; in comparison with the quick administration rate when the dropper bottle was squeezed with more force to achieve 10 or 20 drops administered with one drop immediately followed by another drop. Then the drops were weighed on an analytical balance (1601 MP8, Sartorius, Göttingen, Germany) and, assuming that the densities of the very low concentration solutions were equal to that of water, the average ($n = 10$ or 20, respectively) drop volume (µl) could be calculated.

In the other, detailed part of the experiments, 10 ml of water or a 0.005%, 0.01%, 0.015% or 0.02% (v/v) solution of BAC, respectively, was filled into a 10 ml plastic bottle fitted either a plastic or rubber dropper tip. To eliminate the influence of the dispensing rate, not series of drops but only an individual drop was dispensed at two different dispensing angles, 90° or 45° from horizontal, respectively, into a glass beaker and weighed immediately. The average volume (µl) of individual drops ($n = 20$) was calculated.

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