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Research paper

Influence of *n*-octenylsuccinate starch on in vitro permeation of sodium diclofenac across excised porcine cornea in comparison to Voltaren ophtha

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Abstract

The influence of different *n*-octenylsuccinate starch (AS) formulations, i.e. AS solutions and an AS stabilized emulsion system, on the in vitro corneal permeation behaviour of sodium diclofenac (DfNa) was investigated and compared to the commercial product Voltaren ophtha (VO). Although saturation concentrations of DfNa achieved with polyoxethylene-35-castor oil (POC), which is the solubilizing additive in VO, are higher than those achieved with AS at varying pH values, it was found that AS solutions yield higher DfNa permeation rates than VO or a POC solution. However, permeation is extremely reduced with rising AS concentrations or AS emulsions. Neither pH value (6.5 or 7.4) nor presence of preservative seem to have an impact on permeation activity. In order to assess possible cytotoxic effects of the preparations investigated, red blood cell haemolysis studies were performed with different preparations containing DfNa. None of the tested AS formulations showed significantly high haemolytic data. On the other hand the high in vitro haemolysis obtained with VO is primarily based on an osmotic effect caused by boric acid.

Keywords: *n*-Octenylsuccinate starch; Sodium diclofenac; In vitro corneal permeation; Haemolysis; Voltaren ophtha

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) have proven to be a good alternative to topical steroids in the treatment of ocular inflammation. Undesired effects of steroids to the eyes include the development of posterior subcapsular cataracts and secondary infections due to an immunosuppressive effect. Further a steroid induced elevation of intraocular pressure very often occurs which is reversible once the steroid application is ceased [1].

The activity of NSAIDs is mainly based on an inhibitory effect on the synthesis of prostaglandins. A number of NSAIDs are approved for ocular application. Flurbiprofen and suprofen are used against intraoperative miosis during cataract surgery, ketorolac is given for seasonal allergic conjunctivitis and diclofenac for post-operative inflammation [1–3]. Ibuprofen and nepafenac have also been reported to be effective in the treatment of ocular inflammation [4,5].

Corneal permeation depends mainly on the drug's molecular size [6,7], on its oil/water partition coefficient [6,8–10] and its degree of ionization [11,12]. Ionizable acidic or basic compounds penetrate corneal epithelium mainly in their un-ionized form, which is more lipid soluble [13,14]. The rate and extent of transcorneal transport is influenced by the fraction of ionized and un-ionized molecules, which in turn depends on the pK_a of the drug and the pH of the formulation [15]. Since most NSAIDs are weak acids ionization at lacrimal fluid pH reduces drug penetration and permeation rates, and thus drug potency. Lowering the pH of the preparation may on one hand positively influence permeation rates and drug stability [16] but on the other hand the solubility of these substances is affected. pH decrease is additionally limited and should be adjusted within a physiologically tolerated pH range, which lies between 5.8 and 11.4 [17], finding an acceptable agreement with the drug's stability optimum.

Aqueous sodium diclofenac (DfNa) solutions are chemically and physically unstable [16]. Therefore the

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commercial product Voltaren ophtha contains polyoxyethylene-35-castor oil (POC) to solubilize 0.1% (w/v) DfNa. According to the manufacturer the solubilizing effect is based on micellization of the drug.

As reported in the literature, DfNa solutions may also be stabilized by the formation of inclusion complexes using cyclodextrins [16,18,19]. Owing to a weaker binding strength between DfNa and the specific cyclodextrin derivative in comparison to POC, DfNa permeation rates can be improved. Solutions with decreased pH values are even more effective due to a higher diclofenac partition coefficient [18]. In vivo experiments in rabbits have shown that ocular absorption is enhanced in presence of tramazoline, an alpha-receptor stimulant [3].

Amphiphilic starch (AS) is a chemically modified waxy maize starch with both hydrophilic and lipophilic surface properties. It is gained by esterification of starch with *n*-octenylsuccinic acid [20]. AS is capable of producing polymer stabilized emulsions while on the other hand it may solubilize poorly soluble drugs [21–23]. Oil-in-water emulsions have proven to be appropriate carrier systems for DfNa [24].

Ophthalmic preparations require an acceptably low toxic behaviour. As described in the literature, the ocular tolerance of amphiphilic substances can be assessed by performing haemolysis studies [18,24–30] which correlate highly with the Draize test and may allow to reduce the number of animals needed for in vivo studies [25]. Reports on red blood cell studies performed with Voltaren ophtha reveal high haemolytic activities, which indicates a very low tolerance [18,21]. It was supposed that the haemolytic effect is caused by the surfactant POC [18].

The primary objectives of the present study were to investigate the influence of AS on corneal in vitro permeation behaviour of DfNa. Permeation studies were performed through excised porcine cornea from different AS preparations, i.e. solutions and an emulsion system. Special focus was put on the variations considering AS concentrations, pH value and presence of preservative. Data were compared to those of a POC solution, a pure buffer solution, Voltaren ophtha and Voltaren ophtha sine (not preserved). All preparations contained DfNa 0.1% in accordance with Voltaren ophtha.

In order to interpret previously obtained results of permeation studies [31], saturation concentrations of DfNa depending on solubilizer type, solubilizer concentration and pH were also determined.

Moreover, an attempt was made to explain the high in vitro haemolysis data achieved with Voltaren ophtha. Besides DfNa and POC Voltaren ophtha eye drops contain boric acid and tromethamine[1] for osmolality and pH adjustment and the preservative thimerosal (manufacturer information). POC, which is also known as Cremophor EL, is often applied in parenteral emulsions as it is well tolerated by human erythrocytes [29]. Various solutions containing different substance concentrations and combinations were screened to find the component or components combination responsible for the high haemolytic effect.

[1] The name of the marketed product following this composition is currently 'Diclo CV', while the unpreserved formulation is still referred to as Voltaren ophtha sine. Both are still provided by the manufacturer mentioned.

2. Materials and methods

2.1. Materials

DfNa was purchased from Synopharm (Barsbüttel, Germany), medium chain triglycerides (MCT 812) and tromethamine from Hüls (Witten/Ruhr, Germany), purified castor oil from Henry Lamotte (Bremen, Germany), polyoxyethylene-35-castor oil (POC, Cremophor® EL) from BASF (Ludwigshafen, Germany), sorbitol from Caesar and Loretz (Hilden, Germany), thimerosal from Synopharm (Barsbüttel, Germany), boric acid and sodium hydroxide from Merck (Darmstadt, Germany). Voltaren ophtha® and Voltaren ophtha® sine (not preserved) were provided by Novartis Ophthalmics (Weßling, Germany). Sodium chloride, potassium dihydrogen phosphate and disodium hydrogen phosphate (all pro analysi), purchased from Merck (Darmstadt, Germany), were used to prepare isotonic phosphate buffer, pH 7.4 (PBS 7.4) and pH 6.4 according to the German Pharmacopoeia (DAB 2001); all substances used were of analytical or pharmacopoeial grade.

Acetonitrile and acetic acid (both HPLC grade) were obtained from J.T. Baker (Deventer, the Netherlands); AS type 100, an emulsifying starch, was supplied by National Starch and Chemical (Manchester, UK); double-distilled water was used for all preparations;

2.2. Experimental methods

2.2.1. Preparation of AS formulations

All preparations investigated contained 0.1% (w/v) DfNa and were preserved with thimerosal 0.004% (w/v) in accordance with Voltaren ophtha. pH values were adjusted to 6.5 and 7.4 using a 0.1 N sodium hydroxide solution. Preparations were isotonized with sorbitol if necessary.

2.2.1.1. AS solutions. AS was used at concentrations of 15 and 20% (w/w) in double-distilled water. Moisture contents were previously determined by thermogravimetry (Thermal Analysis System SSC 5200, Software: MAS 5700 MA-Station Version 3.2, SSC 5200H Disk-Station Version 3.2, Version/Type: DSC 220C, Seiko Instruments, Tokyo, Japan). The investigated POC solution contained 5% (w/w) solubilizer.

2.2.1.2. AS emulsion. An oil-in-water (o/w) emulsion (10% (w/w) oil phase) stabilized with 15% (w/w) AS 100 was

prepared. The oil phase consisted of MCT 812 and castor oil (1:1). AS was fully dissolved in cold water and added in a stepwise manner to the oil phase using an Ultra-Turrax (Janke and Kunkel, Staufen, Germany). The pre-emulsion was passed through a high pressure homogenizer (Niro Soavi, type: Panda, Parma, Italy) six times at room temperature applying a pressure of 400 bar. The stock emulsion (AS 100 22.5% (w/w), and lipophilic phase 15% (w/w)) was mixed with a DfNa solution adjusting to a drug concentration of 0.1% (w/v) and homogenized again to achieve a submicron emulsion ($D_{0.9}$ below 1 μm). Particle size distribution was analysed by laser diffraction (Mastersizer MS 20, Malvern, Worcs, UK) and calculated by Malvern SB 09 software using the Mie approximation. pH was adjusted to 6.5 since a breaking of the emulsion was observed at pH values higher than 7 [21].

2.2.1.3. Osmolality measurements. Osmotic activities of investigated preparations were analysed by freeze point measurements (Halbmikroosmometer, Knauer KG, Berlin, Germany; the apparatus was calibrated with a sodium chloride solution (400 mmol/kg) and bidistilled water (0 mmol/kg)) and vapour pressure measurements (Dampfdruckosmometer type: No 11.00, Knauer KG; the apparatus was calibrated with sorbitol solutions within the concentration range of 3.0–8.0% (w/w); the correlation coefficient obtained was > 0.999). The osmolalities of all preparations tested in permeation experiments, excluding PBS pH 7.4, are listed in Table 1.

2.2.1.4. Viscosities. Viscosities were determined using a capillary viscosimeter (Ubbelohde viscosimeter) at a temperature of 20 °C.

2.2.2. Permeation experiments through excised porcine cornea

2.2.2.1. Preparation of diffusion apparatus. Prior to the experiments the corneas were carefully removed from the freshly enucleated pig eyes by incising the sclera circularly at approximately 2 mm from the corneal rim and immediately stored in PBS 7.4. The bulbi were transported in a safely closed plastic bag to avoid dehydration at approximately 4 °C and prepared within 1 h of death. Permeation experiments were carried out with modified Franz diffusion cells [32]. The excised cornea was positioned on the receptor half-cell facing it with the endothelial surface. A small amount of silicon paste was placed on the ground glass below the cornea to prevent leakage and a polycarbonate filter TMTP 5 μm (Millipore, Eschborn, Germany) was put underneath for higher stability. The donor medium consisted of the investigated formulation while PBS 7.4 was used as receptor medium. The cells used had a diffusion area ranging from 0.1963–0.2376 cm^2 and a receptor volume varying between 5.8 and 9.2 ml. The cells were kept at 37 °C in a water bath and the receptor solutions were vigorously stirred with magnetic stirring bars. Samples of 250 μl were taken from the receptor compartment over 20 h to obtain correlation coefficients of 0.99 for all permeation profiles. Permeation profiles which showed a sudden increase in permeation behaviour or correlation coefficients < 0.99 were disregarded due to a possible loss of corneal tissue integrity throughout the experiment. The sampled volumes were replaced by fresh PBS pH 7.4. Permeated DfNa was analysed by UV-HPLC at 276 nm. Permeation coefficients were calculated according to Ref. [33].

2.2.2.2. HPLC conditions. The HPLC system from Waters (Milford, MA, USA) consisted of a '486' tuneable absorbance detector, a '712 plus' autosampler and two 515 HPLC pumps. Separation was achieved with an analytical Hypersil ODS (particle size 5 μm) column (125 × 4 mm) from Grom (Herrenberg, Germany). The analytical Software Millenium 32 from Waters was used to determine the peak sizes of the permeated amounts of DfNa. The mobile phase consisted of double-distilled water/acetonitrile/acetic acid (50:50:2). The flow rate was 1.6 ml/min and DfNa was monitored spectrophotometrically at 276 nm. Linear correlation between peak area and DfNa concentrations was obtained within the concentration range of 0.05–25 μg/ml. The correlation coefficient was 0.999.

Table 1
Permeation coefficients and osmolalities ($n = 3$) of different preparations containing sodium diclofenac 0.1% (w/v)

	Permeation coefficient[a] P (cm/s) × 10^{-6}	n	Osmolality[a] (mmol/kg)
Voltaren ophtha	1.17 ± 0.25	9	310 ± 4.6
Voltaren ophtha sine	1.33 ± 0.15	4	308 ± 3.6
AS 100 solution 15% (w/w) with thimerosal, pH 7.4	2.51 ± 0.28	4	310 ± 4.0
AS 100 solution 15% (w/w) without thimerosal, pH 7.4	2.75 ± 0.34	4	324 ± 5.3
AS 100 solution 15% (w/w), pH 6.5	2.37 ± 0.10	5	326 ± 7.1
AS 100 solution 20% (w/w), pH 7.4	1.11 ± 0.10	6	433 ± 9.8
AS 100 emulsion 15% (w/w), pH 6.5	1.24 ± 0.11	3	320 ± 5.6
POC solution 5% (w/w), pH 7.4	1.63 ± 0.09	6	299 ± 2.5
PBS, pH 7.4	9.63 ± 0.83	3	

[a] Each value represents mean ± SD.

2.2.3. Red blood cell haemolysis studies

Red blood cells were obtained from human blood (27-year-old female with common blood chemistry) by centrifugation (5 min, 1000 × *g*). After the supernatant plasma was removed the erythrocytes were washed three times with PBS 7.4 in order to remove serum proteins. Using PBS 7.4 an erythrocyte stock dispersion with a fixed haemoglobin concentration was prepared. The stock dispersion was refrigerated and kept no longer than 24 h. One hundred microlitres of the stock dispersion were added to 1000 μl sample, well shaken and incubated at 37 °C for different time periods (15 min, 30 min, 1 h). The samples were shaken every 5 min. After centrifugation (3 min, 750 × *g*), to remove intact erythrocytes and debris, 100 μl of the supernatant were added to, 2000 μl of an ethanol/HCl mixture (40 parts of ethanol (99% (v/v) and 1 part of 37% (w/v) hydrochloric acid) and centrifuged again (3 min, 1000 × *g*). The ethanol/HCl mixture avoids haemoglobin precipitation [29]. The absorption of the subsequently achieved supernatant was measured by spectrophotometry at 398 nm against blank samples (ethanol/HCl mixtures containing the same amounts of AS and drug as the samples).

Results were set in relation to control samples of 0% lysis in PBS 7.4 and 100% lysis in double-distilled water. Total haemolysis must show an absorption of about 2.0 ± 0.2 in order to obtain linearity in absorption concentration dependence.

2.2.4. Solubility studies

To investigate saturation concentrations of DfNa, AS 100 and POC solutions with increasing solubilizer amounts were prepared (AS 100: 2, 4, 6, 8 and 10% (w/w), POC: 0.1, 0.5, 1, 5% (w/w)). Surplus amounts of DfNa were added to the solutions and stirred for 48 h at a temperature of 20 °C on a magnetic stirrer. After centrifugation DfNa concentrations of the supernatant were measured by UV detection at 276 nm. The studies were performed at pH 6.5 (PBS 6.5, phosphate buffer 6.4 prepared according to the German Pharmacopoeia, adjusted to pH 6.5 with HCl) and 7.4 in phosphate buffer solutions.

3. Results and discussion

3.1. In vitro permeation studies

Fig. 1 shows that AS solutions seem capable of promoting corneal DfNa permeation activity when compared to permeation profiles of Voltaren ophtha. In order to exclude a possible permeation enhancing effect caused by the preservative, solutions with and without thimerosal were tested. As demonstrated in Fig. 1 and Table 1 showing permeation profiles and permeation coefficients, neither in the case of an AS solution 15% (w/w) pH 7.4 nor in the case of Voltaren ophtha did thimerosal influence the permeation

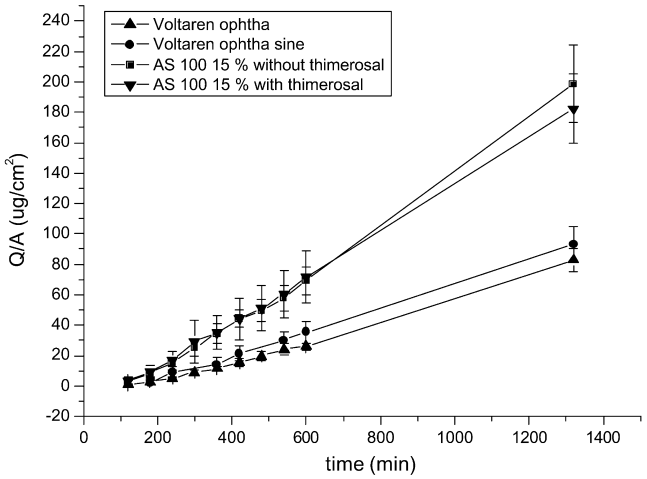

Fig. 1. Permeation profiles of preserved and unpreserved AS 100 15% (w/w) pH 7.4 and Voltaren ophtha.

activity of DfNa. These findings are in agreement with results reported on the ocular tolerance of preservatives [34,35]. In vivo experiments on murine cornea showed that a 0.01% thimerosal solution is not more damaging than a physiological saline solution [34].

The permeation profiles in Fig. 2 reveal that the highest permeability is visibly reached with solutions of DfNa in pure phosphate buffer pH 7.4. A 15% (w/w) AS 100 solution shows higher corneal permeability than Voltaren ophtha. However, permeation activity is greatly reduced with rising AS 100 concentrations, as the profile for a 20% (w/w) AS 100 solution shows (Fig. 2). To a certain extent a higher viscosity is responsible for smaller diffusion speeds. Nevertheless, AS 100 with a concentration of 20% (m/V), which reveals a dynamic viscosity of 48.2 mPa s, shows permeation data comparable with Voltaren ophtha or a POC solution 5% (w/w) (Fig. 2). Both reveal viscosity values around 1 mPa s. This indicates that drug permeation is also influenced by interactions between drug and solubilizer.

Solubility profiles (Fig. 3) point out the solubilization capacities of AS and POC at different pH values and concentrations. POC leads to comparably high saturation concentrations. AS only increases solubility at pH 6.5 while AS seems to lose solubilizing qualities at pH 7.4. This agrees with the loss of emulsifying properties at pH values exceeding 7 [21]. Another cause for reduced interactions may be a greater repulsive effect due to negatively charged octenylsuccinate side chains and also negatively charged DfNa at higher pH values.

Reports dealing with corneal DfNa permeation have shown that permeation data correlate with binding forces between drug and complexing or solubilizing agents [18]. Data obtained with AS also point out that permeation is generally decreased in presence of a solubilizing agent, in accordance with results achieved with complexing agents [18].

A higher solubilizing ability, as in the case of POC, and

higher solubilizer concentrations lead to diminished permeation activities. In the case of POC the solubilizing mechanism is based on a micellization of the drug while the mechanism of interaction between AS and DfNa is still unknown. AS may form inclusion compounds; however, the interactions seem to be weaker than those achieved with POC.

The permeation coefficients (Table 1) further show that incorporation of DfNa into an emulsion system also reduces the drug's permeation capacity. This can be explained by a higher viscosity of the system as compared to the pure starch solution. But since DfNa itself is an amphiphilic compound it may as well be integrated to the system's interface, which can lessen the ability of DfNa to permeate through the physiological membrane due to a reduced concentration gradient of the free drug.

Although an improved permeability was expected for pH 6.5 due to a higher lipophilicity of acidic substances at lower pH values [18], the permeation coefficient does not significantly differ from that achieved at pH 7.4 [30]. This can be due to a pH-dependent change in the AS configuration linked with the changes in solubilizing capacities (Fig. 3). A lower pH should promote permeation but since AS seems to be a stronger solubilizer at pH 6.5, a more rapid permeation of DfNa is hindered.

3.2. Haemolysis studies

None of the investigated AS 100 preparations including a pure 0.1% (w/v) DfNa solution show haemolytic data higher than 1%, whereas Voltaren ophtha shows very high haemolysis data as already reported in the literature [18,21].

It was supposed that the haemolytic effect of the commercial product is caused by the surfactant contained but, as indicated in Fig. 4, the haemolytic activity of Voltaren ophtha becomes smaller when sorbitol 4% (w/w) is added. This is an indication of a mere osmotic haemolysis.

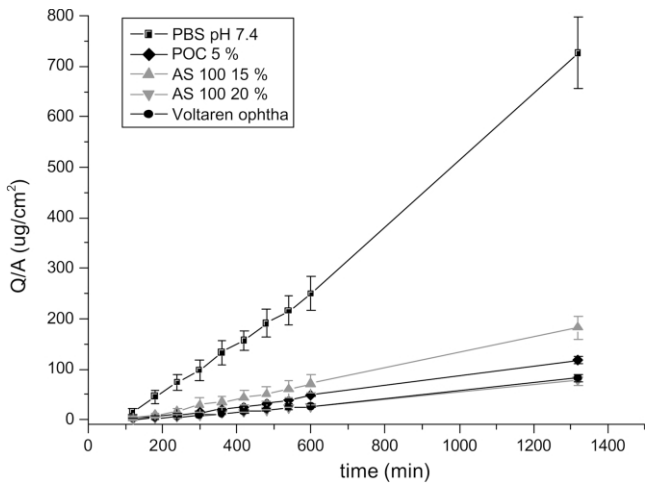

Fig. 2. Permeation profiles of sodium diclofenac solutions containing different amounts of solubilizer.

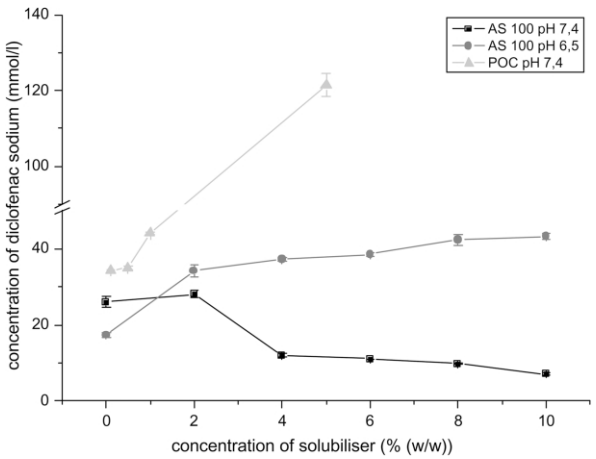

Fig. 3. Saturation concentrations of sodium diclofenac versus solubilizer concentration ($n = 3$).

Additionally, POC solutions of 1, 2.5 or 5% (w/w), isotonized with sorbitol and adjusted to pH 7.4, yield no markedly high haemolysis (Fig. 4).

In order to assess the component(s) in Voltaren ophtha which are responsible for the high haemolysis data, the preparations listed in Fig. 4 were tested. It can be seen that an isotonized POC solution (Fig. 4, columns 3–5) does not show significant haemolytic potential. The high haemolytic activity of boric acid (Fig. 4, column 1) is also lowered immensely when the solution contains sorbitol. Columns 6 and 7 in Fig. 4 reveal the same: in the presence of sorbitol the haemolysis of a POC/boric acid mixture is diminished.

Red blood cells show high haemolytic data when mixed with isotonic amounts of boric acid. This is due to the uptake of boric acid by the erythrocyte. If there is no additional isotonizer (sorbitol) the osmotic activity drops to hypotonic values, water flows into the cells followed by their breakdown. The erythrocyte membrane is not impermeable to all substances such as urea or boric acid [36]. This effect has also been described in articles dealing with parenteral emulsions. Emulsions isotonized with glycerol (2.5% (w/w)) show high haemolytic activities when incubated with red blood cells, while glycerol did not have any lytic properties when added to an already isotonic system [36].

Adding 0.1% (w/v) DfNa to all solutions, replacing sorbitol by sodium chloride or adjusting pH with sodium hydroxide instead of tromethamine led to the same results already pointed out in Fig. 4 (data not shown).

3.3. Conclusions

The high in vitro haemolysis found with Voltaren ophtha is primarily based on an osmotic effect due to the erythrocyte's membrane uptake of boric acid which serves as an isotonizing substance. In this case the in vitro

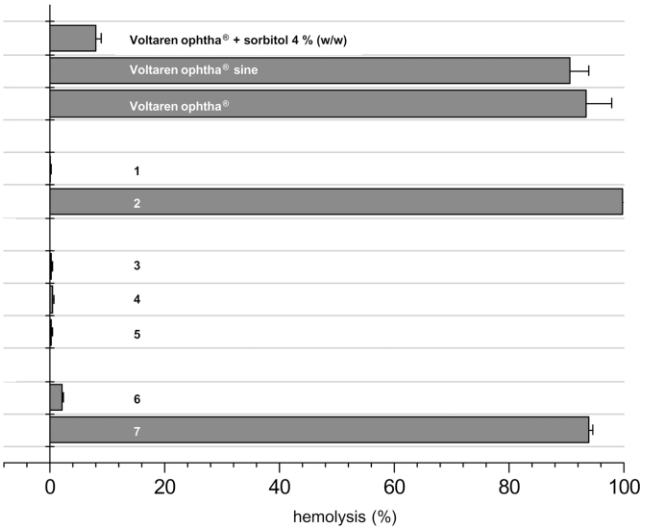

Fig. 4. Haemolytic activities of Voltaren ophtha, Voltaren ophtha sine and Voltaren ophtha containing sorbitol 4% (w/w) in comparison to different preparations after 60 min incubation at 37 °C ($n = 3$). 1, boric acid 1.857% (w/w), sorbitol 5% (w/w), pH 7.4, adjusted with tromethamine; 2, boric acid 1.857% (w/w), pH 7.4, adjusted with tromethamine; 3, POC 1% (w/w), sorbitol 5% (w/w), pH 7.4, adjusted with NaOH 0.1 N; 4, POC 2.5% (w/w), sorbitol 5% (w/w), pH 7.4, adjusted with NaOH 0.1 N; 5, POC 5% (w/w), sorbitol 5% (w/w), pH 7.4, adjusted with NaOH 0.1 N; 6,POC 5% (w/w), boric acid 1.857% (w/w), sorbitol 5% (w/w), pH 7.4, pH adjusted with NaOH 0.1 N; 7, POC 5% (w/w), boric acid 1.857% (w/w), pH 7.4, adjusted with NaOH 0.1 N.

haemolysis test is no reliable method to evaluate the ocular irritation of Voltaren ophtha.

Haemolysis investigations respecting the toxic behaviour of the investigated DfNa-AS systems reveal that these formulations do not significantly affect the human erythrocyte. As compared to Voltaren ophtha, AS indirectly supports the permeation activity of DfNa. It could be seen that the permeated amount depends on concentrations and solubilizing abilities of the solubilizer used. The highest permeation activity can be reached with solubilizer-free systems. Perfusion speed is greatly reduced when a solubilizer is added.

Although AS does not solubilize DfNa as efficiently as POC, it leads to higher permeation values due to a quicker release of the drug from the drug–polymer complex. Since pH, within the tested range, does not greatly influence permeation behaviour, an eye drop formulation containing AS adjusted to pH 6.5 is proposed due to higher saturation concentrations reached for DfNa at this pH value.

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