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Research paper

New surface-active polymers for ophthalmic formulations: evaluation of ocular tolerance

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Abstract

Two *n*-octenylsuccinate starch (AS) types of unknown molecular weights were assessed for ocular tolerance. Irritation potential of different solutions (containing 2 and 15% (w/w) AS) and AS stabilized emulsions (containing 15% (w/w) AS) was evaluated in vivo in rabbit eyes, using a confocal laser scanning microscope, and in vitro on treated excised pig corneas by light microscopy of histological cross sections. Both AS types were previously characterized by viscosity, osmolality and surface tension measurements. All tested solutions and emulsions showed good eye tolerance regardless of concentration and emulsifying properties suggesting AS to be a good alternative to commonly used solubilizing or emulsifying agents in ophthalmic formulations.

Keywords: *n*-Octenylsuccinate starch; Ocular tolerance; Confocal laser scanning microscopy; Cornea histology; Ophthalmic formulations

1. Introduction

Polymeric hydrogels have been widely used to increase viscosity and consequently retention time of eye drops on the ocular surface in order to increase intraocular drug levels. The most widely used polymers include cellulosic derivatives, poly(vinyl alcohol), sodium hyaluronate, and carbomer [1]. Recently, the use of starch derivatives has been described for the formulation of anti-inflammatory eye drops [2]. Chemical modifications of starch, including oxidation, esterification, etherification, hydrolysis and dextrinization, allow adaptation of the hydrophilic properties of polymers to the physical chemical characteristics of the particular drugs [3].

Amphiphilic starch of the *n*-octenylsuccinate starch type (AS) is a chemically modified waxy maize starch gained by substituting hydrophilic starch moieties by lipophilic *n*-octenylsuccinic acid groups [4,5]. Consequently, the starch acquires emulsifying properties due to feasible formation of hydrophobic–hydrophobic interactions. Despite these modifications, biodegradability of the starch is maintained [5]. AS has proven to be useful in the development of degradable plastics composed of AS and hydrophobic plastics [6,7]. However, substituted starch shows higher resistance to enzymatic degradation [8].

AS is used in the food and cosmetic industry [9,10] and is approved by the FDA as a food additive with the stipulation that the octenylsuccinate content does not exceed 3% [11]. AS is an economically interesting alternative to traditional stabilisers such as spray dried gum acacia, vegetable proteins, lecithin, or gelatin which are associated with problems of uncertain supply and fluctuating price [5,12]. AS is highly soluble in cold water and produces low viscosities with high solid content [13] which is of major importance in encapsulation technology.

AS sodium salts especially perform as stabilisers for beverage emulsions and as encapsulation carriers for oxidizable substances useful in nutritional supplementation [5,14,15]. On the other hand, AS aluminium salts are highly

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hydrophobic and can be used as a talc replacement in body powders to adsorb moisture or excess oil from the skin.

Thus, AS might also be beneficial for certain aspects of pharmaceutical formulation. As reported in the literature, low substituted AS increases dissolution rates in solid dosage forms [16]. Associations of AS with xanthan-gum have revealed advantages in the formulation of hydrophilic sustained release matrices [17] and properties of crystal growth inhibition in sucrose ester suspensions [18]. Sodium diclofenac, incorporated in an AS solution, has shown improved corneal permeation behaviour as compared to tested marketed eye drop preparations [2,19].

Due to AS's emulsifying properties, it is mandatory, when its use is intended for the ocular route, to assess in vivo its ocular tolerance. Indeed, surfactants or preservatives with surfactant properties used in eye drops are known to cause varying ocular irritations by mechanisms such as association with biological membranes resulting in changes to surface charge, alteration of barrier functions, or denaturation and cell death [20,21]. Currently, the Draize rabbit eye test is used to assess ocular irritancy. However this methodology has been criticized for its lack of objectivity and sensitivity. More recently, alternatives have been proposed to replace the Draize test including: light microscopy and confocal microscopy. These approaches have been shown to objectively quantify surfactant-induced eye irritation in the rabbit [22].

The aim of the present study was to compare, with respect to their ocular tolerance, different AS types of unknown molecular weights and diverse emulsifying properties. Both types, AS 100 and AS 300, provided by different manufacturers, are identical in chemical structure but may differ in degree of substitution (ds), which does not exceed 3%. They are won from waxy maize starch, which is chemically modified and hydrolysed, by controlling the viscosity, to reduce the molecular weight of the starch molecule. The investigated AS types mainly differ in molecular weight and molecular weight distribution. Since, it was found that increasing the ds does not necessarily improve the emulsifying properties of AS [9] both AS types were characterized by performing viscosity, vapour pressure and surface tension measurements in order to gain information on molecular weight and molecular weight distribution, which greatly influence emulsion formation and stability [23]. To evaluate the preparations' irritation potential, in vivo ocular tolerance tests were carried out in rabbit eyes. After applying AS solutions and emulsions at different concentrations to the animals' corneas, tissue damage was assessed using a confocal laser scanning microscope following fluorescein labelling. Additionally, the tested formulations were incubated with excised porcine corneas. Histological cross sections of treated material were evaluated by light microscopy for pathological modifications caused by an irritant substance.

2. Materials and methods

2.1. Materials

Medium chain triglycerides (MCT 812) and thimerosal were purchased from Synopharm (Barsbüttel, Germany), sorbitol from Hänseler AG (Herisau, Switzerland) and sodium hydroxide from Amtech-Chimie SA (Lausanne, Switzerland). Sodium fluorescein was obtained from Reactolab (Servion, Switzerland). 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) according to the German Pharmacopoeia (DAB 2001); all substances used were of analytical or pharmacopoeia grade.

AS type 100 and type 300, both emulsifying starches, were supplied by National Starch and Chemical (Manchester, United Kingdom) and Roquette frères (Lestrem, France), respectively. Double-distilled water was used for all preparations.

2.2. Experimental methods

2.2.1. Characterisation of AS types

Flow measurements. Rheological properties were assessed using a rheometer CVO (Bohlin Instruments, Mühlacker, Germany) equipped with a concentric cylinder measuring geometry C25. Viscosity data were derived from the linear region of the flow profile (shear rate 50–300 l/s, $n = 50$). AS solutions were prepared (10, 15, 20, 25, 30, 35 and 40% (w/w)) by dissolving AS in double-distilled water. Two o/w-emulsions containing 10% (w/w) MCT 812 stabilized with AS 15% (w/w) were also characterized. Each formulation was measured at 20 °C three times.

Osmolality. In order to compare the AS types, the osmotic activities of AS solutions of different concentrations (10, 15, 20 and 25% (w/w)) were measured. Osmotic activities of investigated preparations were analysed by vapour pressure measurements (vapour pressure osmometer type: No 11.00, Knauer KG, Berlin, Germany; 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).

Surface tension. Surface tension measurements were carried out with a thermostatically-controlled Processor Tensiometer K100 (Krüss GmbH, Hamburg, Germany) provided with a Du Noüy ring (ring radius: 9.545 mm, wire diameter: 0.37 mm) at 20 °C. The apparatus was calibrated with double-distilled water achieving a surface tension of 72.14 mN/m. The reduction of surface tension caused by AS 100/300 15% (w/w), both adjusted to pH 6.5 with 0.1 N-NaOH, was measured in triplicate.

2.2.2. Tolerance evaluation

Preparation of AS formulations. The AS solutions (AS S) investigated contained 2 and 15% (w/w) AS of both types.

AS 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/Typ: DSC 220C, Seiko Instruments, Tokyo, Japan). AS emulsions (AS E) were stabilized with 15% (w/w) AS 100/300. The lipophilic phase consisted of MCT 812. Oil-in-water (o/w) emulsions (10% (w/w) oil phase) were prepared by dissolving AS in cold water and adding it to the oil phase using an Ultra-Turrax (Janke and Kunkel, Staufen, Germany). The pre-emulsions were passed through a high pressure homogeniser (Niro Soavi, type: Panda, Parma, Italy) six times at room temperature applying a pressure of 400 bar (AS 100) and 300 bar (AS 300) to achieve submicron emulsions ($D_{0.9}$ around 1 μm). Particle size distribution was analysed by laser diffraction (Mastersizer MS 20, Malvern, Worcs, United Kingdom) and calculated by Malvern SB 09 software using the Mie approximation.

pH values of all formulations tested were adjusted to 6.5 using a 0.1 N sodium hydroxide solution as AS loses its emulsifying properties at pH values higher than seven, where emulsions tend to break quickly [23]. Osmolality was adjusted with sorbitol using a calibrated vapour pressure osmometer (Wescor 5500, Baumann-Medical, Wetzikon, Switzerland) and ranged between 280 and 330 mmol/kg.

All preparations were preserved with thimerosal 0.0015% (w/w).

Confocal laser scanning microscopy. New Zealand albino rabbits of either sex, weighing between 4 and 5 kg were separately kept in an air-conditioned, illumination-controlled room at 19 °C ± 1 °C and a relative humidity of 50 ± 5%. They were fed a standard pellet diet and water ad libitum. All animals were healthy and free of clinically observable abnormalities.

The experiments of the present investigation were run in accordance with the Association for Research in Vision and Ophthalmology (ARVO) resolution on the humane handling of animals in ophthalmic and vision research and were approved by the local ethics committees for animal experimentation.

Millilitres of each tested formulation were repeatedly applied onto the rabbit's right cornea throughout 3 days, every 2.5 h four times per day and once on the fourth day right before the microscopy experiment

The fluorescent images of the treated rabbit corneas were visualised by CLSO using a previously described optical device [20]. The total areas of corneal lesions were quantified by an image processing system [20] and expressed in percent of the total corneal surface. Data were statistically compared applying the Student's *t*-test (unpaired samples). A probability level of 0.05 was chosen for all comparisons. Calculations were made with a Microsoft Excel 7.0 program.

Light microscopy of histological preparations. To examine the influence on corneal structure and integrity, cornea was removed from fresh pig eyes [2] and incubated at 37 °C for 2 h in the AS formulations. PBS and a sodium dodecylsulfate (SDS) solution in PBS 0.1% (w/w) were taken as references. Corneas were incubated for 0.5 h in SDS 0.1% (w/w).

After incubation, the corneas were washed with PBS, and immediately fixed with a formalin solution 8% (w/w). The material was dehydrated with an alcohol gradient, put in melted paraffin and solidified in block form. Cross sections (< 1 μm) were cut, stained with haematoxyline and eosine (H and E), blinded and microscopically observed for pathological modifications in co-operation with a pathologist ($n = 3$).

3. Results and discussion

3.1. Characterisation of AS types

Fig. 1 displays for both AS types tested an increase in viscosity with concentration. AS type 100 shows higher viscosities than AS 300. Higher viscosities are an indication of the presence of rather long chain AS molecules. Both AS types produce solutions revealing Newtonian flow behaviour, which can be drawn from a linear dependence between applied shear rate and shear stress (results not shown here).

Depending on synthesis conditions, e.g. extent of starch hydrolysis, differently manufactured AS types may vary in viscosity and osmotic activity. Both characteristics may provide indications about the polymer's molecular weight and its molecular weight distribution.

On the other hand, Fig. 2 shows that the AS types investigated differ in their osmotic properties. AS 100 reveals a stronger osmotic activity than AS 300 which may be caused by a higher percentage of rather short chain molecules or, respecting the higher viscosities of AS solutions, a broader molecular weight distribution.

AS 100 at a concentration of 15% (w/w) also lowers surface tension of water by a greater value, 34.2 mN/m, than

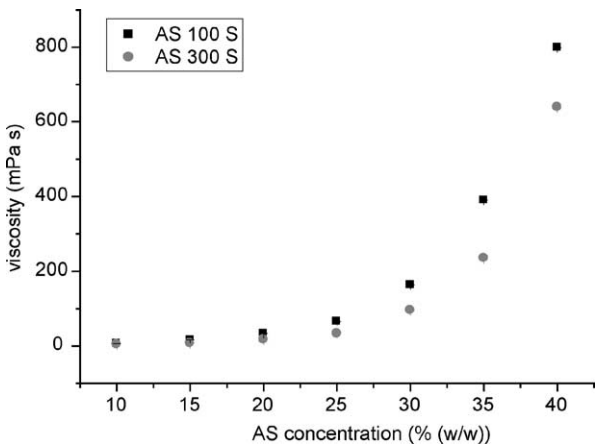

Fig. 1. Viscosities of different AS type solutions (S) depending on concentration ($n = 3$), mean ± SD, symbol includes standard deviation.

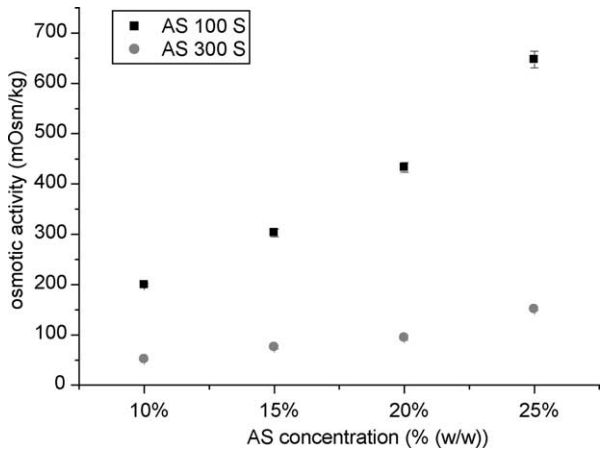

Fig. 2. Osmotic activities of different AS type solutions (S) depending on concentration ($n = 3$), mean ± SD, symbols include standard deviation.

AS 300 which is 28.6 mN/m. As already reported, AS 100 under chosen conditions promotes the formation of finer emulsions within shorter homogenisation times. This means that $D_{0.9}$ values around 1 μm and below can readily be achieved. Additionally emulsions composed with AS 300 tend to destabilize more quickly [23]. A higher viscosity along with a higher presence of short chain molecules, as in the case of AS 100, seems to yield more stable emulsions as already reported in the literature [23].

3.2. Tolerance evaluation

The extent of corneal surface damage caused by repeated instillation of AS preparations is shown in Fig. 3. Two different AS types with characteristics mentioned above

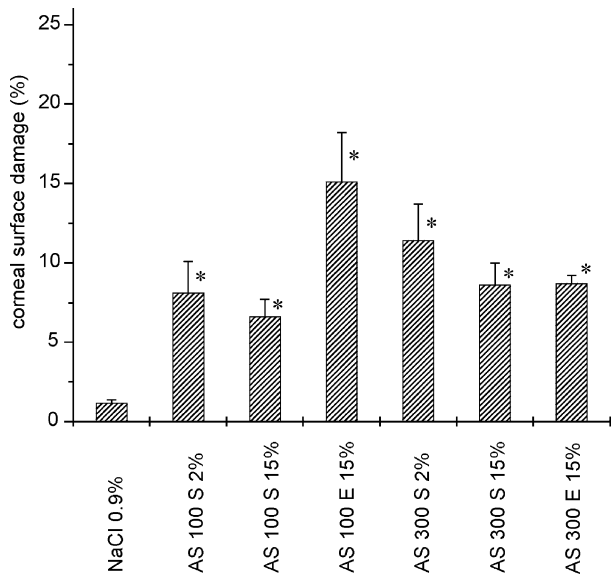

Fig. 3. Extent of corneal surface damage (%) in rabbits caused after instillation of various AS formulations (S, solution; E, emulsion) compared to a sodium chloride solution (0.9%). Mean value ± SD ($n = 3 - 4$), Student's t-test (unpaired samples), $^*P < 0.05$ (significant difference).

Table 1
Characteristics of different AS formulations (S, solution; E, emulsion) assessed for ocular tolerance

		Osmolality (mOsm/kg)	Viscosity (mPa s)
AS 100	S 2%	299[a]	n.m.[b]
	S 15%	326	15.9
	E 15%	320	40.0
AS 300	S 2%	282[a]	n.m.[b]
	S 15%	299[a]	7.4
	E 15%	308[a]	21.2

[a] Osmolality adjusted with sorbitol.
[b] Not measured.

were tested in comparison to data achieved with a physiological sodium chloride solution [24]. Solutions (S) containing 2 and 15% (w/w) AS and emulsions (E) stabilized with 15% (w/w) AS were evaluated for ocular tolerance (Table 1).

Due to a detectable surface activity, all tested AS systems showed significantly higher corneal surface damage, ranging from 5 to 20%, as compared to a physiological saline solution (Fig. 3), which causes about 2% of damaged corneal surface. These damaged areas in the case of a saline solution are a result of physiological desquamation observed even in the healthy eye [25]. Nonetheless, the total of fluorescent areas with AS treatment, representing harmed corneal tissue, never exceeded 19% (Fig. 3). This indicates, according to a previously established scale [26], a good tolerance for all AS preparations respecting the tested concentrations.

With regard to solutions, Fig. 3 reveals that raising the AS concentrations from 2 to 15% does not necessarily lead to a greater damage of corneal area. Both polymer types, AS 100 and 300, showed no significant differences. When comparing S 2% with S 15%, no significant increase of irritation is observed. In contrast, results obtained with preservatives and absorption enhancers have demonstrated the influence of the agents' quantity on corneal injury [24,25].

Although, AS 100 has more pronounced surface properties than AS 300, drawn from data of surface tension measurements and emulsion formation and stability [23], both types reveal no notable differences when instilled in equal concentrations independent of the sort of formulation. The degree of polymerisation, or in terms of starch derivatives the dextrose equivalent value, seems to have an effect on emulsifying properties but not on the extent of ocular irritation.

Emulsions as carrier systems for ocular administration offer the possibilities of incorporating lipophilic or difficult-to-solubilize and -stabilize drugs, such as sodium diclofenac [27]. Ophthalmic emulsions with higher viscosities may extend pre-ocular retention times while, on the contrary, often a temporary blurred vision appears. Reports on the haemolytic behaviour of surface active agents have shown that erythrocyte lysis could be significantly decreased when

the lytic agent was incorporated into an emulsion system [28]. This membrane protective effect described [28] could not be confirmed in eye tolerance evaluation by confocal laser scanning microscopy. While the effects caused by an AS type 300 solution and emulsion (S/E 15%) are comparable, AS 100 visibly imparts significant differences between S and E 15% (Fig. 3). Fig. 4a–c show fluorescent images of three different AS preparations, S 2 and 15% and E 15%. Damaged cornea tissue occurs as bright regions resulting in a total of 7.45% (Fig. 4a), 8.5% (Fig. 4b) and 15.1% (Fig. 4c).

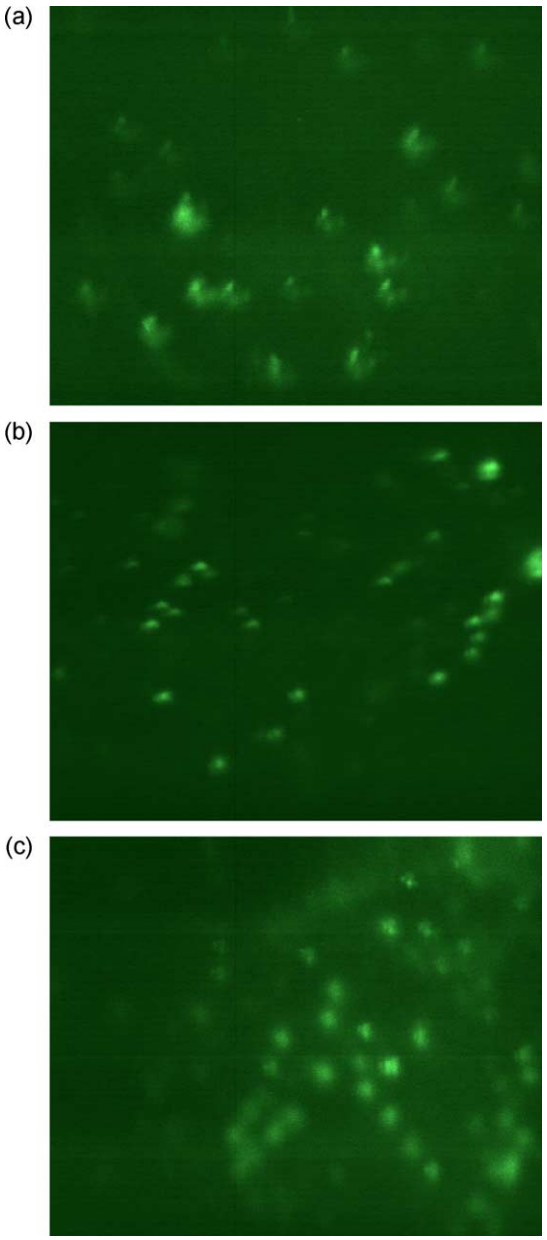

Fig. 4. Fluorescent images of rabbit corneas illustrating corneal lesions (bright spots) after repetitive instillation of 3 different AS 100 preparations (S, solution; E, emulsion). (a) AS 100 S 2% (w/w) represents 7.45%, (b) AS 100 S 15% (w/w) represents 8.5%, (c) AS 100 E 15% (w/w) represents 15.1% damaged surface area.

AS emulsions show, like AS solutions, Newtonian flow behaviour, while AS 100 E 15% produces a comparably high viscosity of 40.0 mPa s (Table 1). On the other hand, emulsions may break on contact with the cornea and lacrimal fluid which leads to higher local emulsifier concentrations. Both effects may explain the increased but still acceptable irritancy.

Fig. 5a–c present corneal cross sections after incubation of freshly excised pig corneas with various

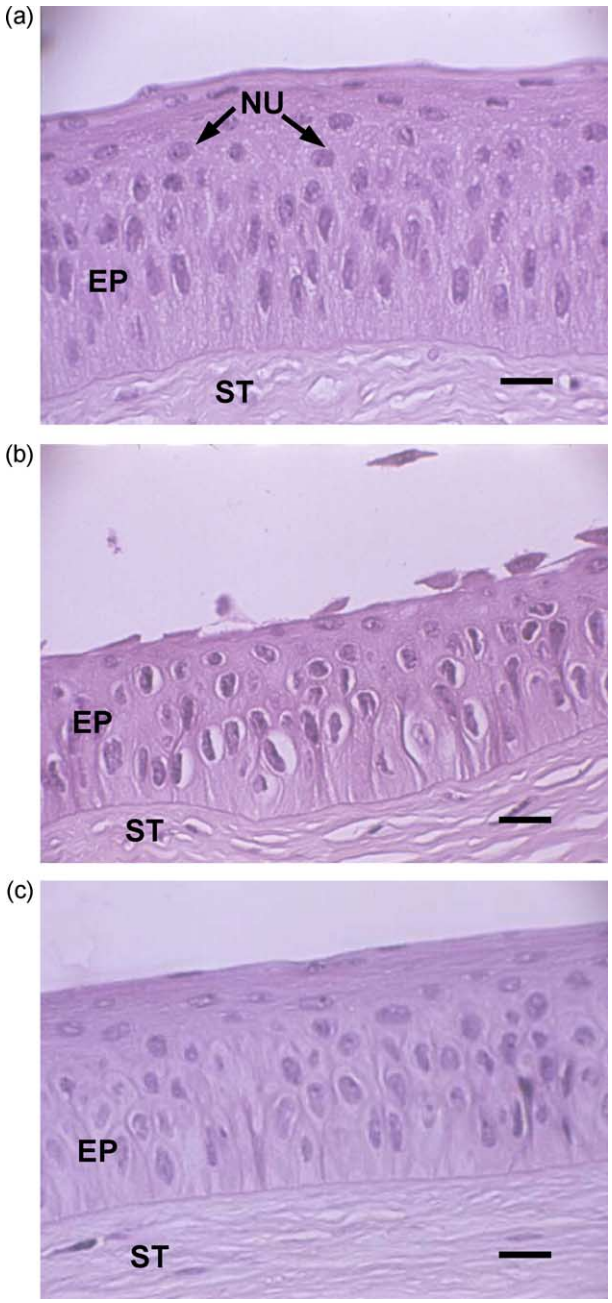

Fig. 5. Histologic cross sections of excised porcine cornea showing epithelium (EP) and stroma (ST), stained with hematoxylin-eosin (scale bar 20 μm) after incubation at 37 °C with; NU, nuclei. (a) Isotonic phosphate buffer (PBS) pH 7.4 (2 h), (b) Sodium dodecylsulfate (SDS) solution 0.1% (w/w) (0.5 h) and (c) AS 100 solution 15% (w/w), pH 6.5 (2 h).

preparations to investigate their influence on corneal cell structure and tissue integrity. After incubation in a physiological phosphate buffer solution pH 7.4 (Fig. 5a) epithelium (EP) and stroma (ST) structure is maintained. Typical stratified epithelial layer can be recognized by the basal columnar cells and the squamous surface cells appearing with a bulge at the nuclei (NU). When corneal epithelium is exposed to an irritant, like SDS (Fig. 5b), previously narrow intercellular spaces are clearly widened, cells and nuclei are deformed and superficial epithelial cells are detached from tissue assembly. Treating corneas with the tested AS formulations as exemplified in Fig. 5c, showing a cornea cross section after incubation in AS 100 S 15%, leaves corneal structure and integrity visibly unaffected.

4. Conclusions

Previously achieved low haemolytic data (data not shown here) indicated a good tissue acceptability of AS types, which has been confirmed in vivo with confocal laser scanning microscopy in rabbit eyes and in vitro with light microscopy of histological cross sections of treated excised pig corneas. When comparing different AS types, varying in degree of polymerisation and molecular weight distribution, higher concentrations or superior emulsifying properties do not necessarily increase ocular irritation. Polymer stabilized emulsions can be an appropriate alternative carrier system for highly lipophilic and poorly soluble ocular therapeutics. Hence, AS is a promising new excipient for ophthalmic formulations due to satisfactory solubilizing and emulsifying properties coupled with a good eye tolerance.

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