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(54) Title: ENHANCEMENT OF ENZYMATIC ACTIVITY IN CLEANING CONTACT LENSES BY THE USE OF HYPOTONIC SOLUTIONS

(57) Abstract

Hypotonic solutions enhance enzymatic activity in proteolytic cleaning of contact lenses. The hypotonic solution may have an osmolality value ranging between 0 and 275 mOsm/kg, but preferably will be between 100 and 200 mOsm/kg.

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ENHANCEMENT OF ENZYMATIC ACTIVITY IN CLEANING CONTACT LENSES BY THE USE OF HYPOTONIC SOLUTIONS

Background

This invention relates to the potentiation of the enzymatic activity in cleaning contact lenses. More specifically, this invention is a method for potentiating the proteolytic activity of proteases in the cleaning of contact lenses by carrying out the cleaning in a hypotonic solution.

Related Art

Contact lenses, particularly those with a hydrophilic surface such as the hydrogel lenses and the hard, gas permeable lenses with a treated surface, encounter protein accretions during normal wear. It is beneficial, if not often times necessary, to remove these accretions in order to maintain visual acuity, prevent eye irritation and to prevent the development of giant papillary conjunctivitis.

The use of proteolytic enzymes has been developed to remove such deposits. See, for example, U.S. Patent 3,910,296, and 4,285,738. The '296 disclosure makes no comment on the effect of tonicity value on enzyme activity. The '738 patent discloses in the specification and stipulates in the claim, that the solution must be hypertonic. Hypertonic is not specifically defined, but the urea concentration is specified as being between 5% through saturation (weight/volume). The normal tonicity

value is that of a physiological solution as illustrated by the 0.9% by weight/volume concentration of aqueous saline.

It has now been found that where the enzymatic solution is made hypotonic, removal of adhered protein by the enzyme is substantially enhanced throughout the effective pH range of the enzyme. Studies were carried out with several enzymes, papain, subtilisin, pancreatin, each of which clearly demonstrated a substantial increase in activity when the solution was made hypotonic.

Summary of the Invention

This invention covers a method for enhancing the activity of an enzyme used in the cleaning of contact lenses, which method comprises carrying out the cleaning regime in a solution having an osmolality value up to about 275 milliosmoles/kilogram.

Specific Embodiments

The use of a hypotonic solution to enhance enzyme activity for cleaning contact lenses is applicable to any proteolytic enzyme which may be used to remove protein from contact lenses.

Tonicity values may range from 0 up to about 275 mOsm/kg. The enzyme itself will be active at tonicity values close to 0, but as a practical matter it is difficult to prepare such solutions because of the solutes normally present in diluents, including purified water. Tests have been carried out where the osmolality was as low as 6 mOsm/kg. A more preferred lower limit is about 50 mOsm/kg, which number allows for the addition of small amounts of salts, stabilizers, enzyme co-factors or other excipients which may be useful and beneficial to solution stability, enzyme activity or the like.

On the upper side, 275 milliosmoles is approximately

the top end of the range so far as enjoying substantially enhanced proteolytic activity is concerned. More preferably, the upper limit will be between 175 and 200 mOsm/kg.

The adjustment of tonicity value can be made with any number of excipients and constituents well known in the art. If an enzyme co-factor is critical or essential to the cleaning process, obviously its presence must be given primary consideration in adding materials to the formulation. Salts of any sort including salts which are necessary co-factors for enzymatic activity such as calcium should be accounted for in formulating these hypotonic solutions. The hypotonic value is determined, that is measured, after addition of the enzyme.

In the following examples, the ability of a given solution and enzyme to remove protein from a contact lens was determined by essentially the same procedure each time. Generically, the procedure was to take a contact lens and coat it with heat denatured lysozyme by placing the lens in a phosphate buffered saline solution to which was then added sufficient lysozyme to make a 0.1% solution by weight. The lysozyme was from egg white. These solutions were then heated for 30 minutes at about 95 C. The lenses were removed, cooled and rinsed with distilled water and viewed to determine what type of lysozyme accretion was on the lens at time zero.

Protein deposit classification (typing): as a means for quantifying the proteolytic activity of enzymes in removing absorbed protein from the lenses, a system was developed whereby the protein on the lenses was visually quantified before and after enzyme treatment.

After a lens had been heated for 30 minutes in the lysozyme solution, the lens was wetted with distilled water, rubbed between the thumb and finger, then grasped by the edge with plastic tweezers and rinsed with

distilled water again. The convex side of the lens was viewed under a microscope at 100X magnification. A film or deposit detected under these conditions was classified according to the percentage of the lens surface covered by the deposit. The protein removal efficacy of an enzyme solution is expressed in terms of the percentage of the lens surface which has been cleaned. This number is derived by subtracting the percentage of the lens surface covered by the protein deposit from 100%.

Example 1

Effect of Osmolality

Twenty-four Hydrocurve® II hydrogel lenses (Barnes-Hind, Inc. Sunnyvale, California) were coated with lysozyme using the standard procedure. Each was viewed after treatment and determined to have at least 98% of its surface covered by a protein film. In most cases, 100% of the lens surface was covered by a protein film. A subtilisin enzyme containing solution was prepared as follows: cysteine hydrochloride monohydrate (1.001g), sodium borate dihydrate (1.911 g), sodium carbonate anhydrous (3.106g), polyethylene glycol 3350 (0.403g), tartaric acid (2.002g), S. carlsberg (0.041g), obtained from Novo Industries of Denmark, was dissolved in 300 ml of purified water, the pH adjusted to 8.4 with sodium hydroxide or hydrochloric acid, and then water added in a quantity sufficient to make 1000 ml. Three 200 ml portions were removed and the osmolality adjusted with sodium chloride and the pH with NaOH or HCl as needed to obtain the figures given in table I.

Three lenses were soaked in each of the solutions for 3 hours at room temperature, then a determination of percentage residual protein deposit and cleaned lens surface made as per the standard procedure described above.

TABLE I - Effect of Osmolality

Average % Lens Surface Cleaned

pH			
9	98.3±2.9	23.3±5.8	11.7±5.8
8.5	100	11.7±2.9	13.3±2.9
7.8	90±17	--------	3.3±2.9
	150	326	420

Osmolality (mOsm/kg)

Table I shows that greater cleaning was observed at 150 mOsm/kg than at either 326 or 420 mOsm/kg.

Example 2

Effect of pH vs. Osmolality

Hydrogel-type lenses (Hydrocurve II®, 55% water, sold by Barnes-Hind, Inc.) were treated with heat-denatured lysozyme as described above.

A subtilisin-A-containing solution was prepared (0.04 mg/ml of water without excipients, activity: 0.0012 Au/ml) in such a manner as to have pH values between approximately 5.0 and 10.0. The osmolality value was then adjusted to 6, 133 and 264 mOsm/kg with NaCl for each of these solutions, each of the tonicity values being tested at pH 9.0. Lenses were then soaked in these solutions (five in each) for 2 hours at room temperature, rinsed, and analyzed for percentage residual protein deposit as described above. Results are given in Table II.

Table II - Effect of pH vs. Osmolality

% Lens Surface Cleaned

pH			
10.0	93.0±4.5		
9.0	69.0±8.2	34.0±5.5	18.0±2.7
8.0	62.0±4.5		
7.0	58.0±4.5		
6.0	23.8±4.8*		
5.0	11.0±2.2		
	6	133	264

Osmolality (mOsm/kg)

* Only four lenses.

It can be seen from the data presented in Table II that both solution pH and osmolality are important parameters asserting the cleaning efficacy of an enzyme solution. Since subtilisin-A is an alkaline protease, having its greatest activity between pH 8 and 10, the greatest cleaning efficacy is observed in this pH range also.

Example 3

Lenses with lysozyme protein deposits covering at least 98% of the lens surface were cut in half, one half being soaked in one enzyme solution and the other in another enzyme solution in the first comparison (pancreatin data); whole lenses were used in the other studies. The enzyme solutions were comprised of enzyme and excipients as indicated in Table III. Lenses were bufilcon-A sold by Barnes-Hind, Inc. under the name Hydrocurve II®. Results from each of the several formulations are listed in the following table. The abbreviation DI is used for deionized water.

Table III

Effect of Varying Osmolality on Enzyme Cleaning Efficacy

Enzyme Used	Diluent	pH	Osmol-ality	Soak Time	Average % Cleaning
Pancreatin[1]	DI water	8.58	175	4 hrs	32.0±25.4
Pancreatin	saline[2]	7.97	382	4 hrs	1.3±2.3
Subtilisin carlsberg 0.04 mg/ml[3]	DI water	8.43	157	3 hrs	98.3±2.9
	water & NaCl	8.43	335	3 hrs	25.6±13.9
Subtilisin carlsberg 0.04mg/ml[4]	DI water	8.86	124	1 hr	79.6±11.3
	saline[5]	8.21	418	2 hrs	16.4±.4.6

1. Alcon Optizyme™ tablet.
2. Normal saline.
3. Same formulation excipients as in Example 1, Table I.
4. A subtilisin enzyme tablet was dissolved in either 10ml of DI water or saline. Each enzyme tablet contained: 30mg N-acetylcysteine, 34mg sodium carbonate, 7mg tartaric acid, 4mg polyethylene glycol 3350, 4mg subtilisin-A (subtilisin carlsberg from NOVO Industries of Denmark), and 50mg of lactose.
5. Allergan Hydrocare™ Preserved Saline.

It can be seen from the data presented in Table III that for both pancreatin and Subtilisin carlsberg, the solutions with the lowest tonicity produced the highest cleaning efficacy.

Example 4

The effect of osmolality values on cleaning efficacy was investigated with papain enzyme. Solutions of 1mg/ml papain, 1mg/ml L-cysteine and 0.8mg/ml EDTA at 95mOsm/kg, 193mOsm/kg and 291mOsm/kg were tested (pH 8.4). Hydrocurve II lenses (55% H_2O) from Barnes-Hind were coated with denatured lysozyme as described in Example 1. Three lenses were soaked in the 95mOsm/kg solution and four lenses in each of the other two solutions. Soaking time was 3.5 hours. Total percent surface cleaned was determined as per Example 1. the results are given in Table IV.

Table IV

Lens	%Surface Cleaned	Mean ± S.D.	Exp. Conditions
A1	100	78±38	1mg/ml papain, 1mg/ml L-cysteine .8mg/ml EDTA pH_f=8.4 osmolality=95mOsm/1kg
A2	35		
A3	100		
B1	60	48±28	Same as above except osmolality = 193mOsm/1kg
B2	30		
B3	80		
B4	20		
C1	30	21±12	Same as above except osmolality = 291mOsm/1kg
C2	20		
C3	5		
C4	30		

It can be seen from the data presented in Table IV that cleaning efficacy increases with lower solution tonicity for papain-containing solutions.

WHAT IS CLAIMED IS:

1. A method for enhancing the activity of an enzyme used in the cleaning of contact lenses, which method comprises carrying out the cleaning regimen in a solution having an osmolality value up to 275 mOsm/kg.

2. The method of claim 1 wherein the osmolality is between 100 and 200 mOsm/kg.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US87/00045

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

INT. CL[4] B08B 11/00
US CL. 134/25.1

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
US	134/25.1, 25.4, 26, 28, 42 252/DIG. 12, 105, 174.12, 525 435/262, 264

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
Y	US, A, 4,521, 254 (ANDERSON ET AL), PUBLISHED 04 JUNE 1985, See cols. 10-11, lines 48-68, and lines 1-5, and lines 15-21, respectively.	1, 2
Y	US, A, 3,910,296 (KARAGEOZIAN ET AL), PUBLISHED 07 OCTOBER 1975, See examples 1-4, cols. 5-6, respectively.	1, 2
Y	US, A, 4,285,738 (OGATA), PUBLISHED 25 AUGUST 1981, See col. 2, lines 7-13.	1, 2
A	US, A, 3,240,709 (RANKIN), PUBLISHED 15 MARCH 1966, See col. 1, lines 13-17, and lines 59-61, respectively.	1, 2
A	US, A, 4,048,122 (SIBLEY ET AL), PUBLISHED 13 SEPTEMBER 1977, See col. 1, lines 51-55.	1, 2
A	US, A, 4,613,380 (CHEN), PUBLISHED 23 SEPTEMBER 1986, See col. 1, lines 1-14.	1, 2
A	US, A, 4,263,054 (GIAMBALVO), PUBLISHED 21 APRIL 1981, See col. 1, lines 20-28.	1, 2

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IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
26 FEBRUARY 1987	06 MAR 1987
International Searching Authority [1]	**Signature of Authorized Officer** [20]
ISA/US	SHARON T. COHEN

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No [18]
A	US, A, 4,626,292 (SHERMAN), PUBLISHED 02 DECEMBER 1986, See col. 2, lines 59-65.	1, 2