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DEPOSITION STUDIES USING MULTIPURPOSE SOLUTION ON HYDROPHILIC CONTACT LENSES

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SUMMARY

In the tears lysozyme and albumin are also present besides other constituents. All these constituents form a biofilm on the hydrophilic contact lenses - minutes after the lens is placed in the eye. These deposits if not removed make the contact lens translucent and impair visual acuity. For the removal of deposit multipurpose solution is used.

In the study, deposits of lysozyme and albumin were made on hydrophilic contact lenses deliberately. These deposits laden contact lenses were then treated with multipurpose solution for 12 hrs. The extent of removal of these deposits by the action of sodium citrate present in multipurpose solution was assessed by measuring albumin and lysozyme quantitatively by using standard analytical procedures.

It was observed that 0.1% of sodium citrate could remove lysozyme and albumin efficiently. Albumin deposited more as compared to lysozyme and non ionic hydrophilic contact lenses are less prone to deposition than ionic. Any further increase in sodium citrate was not desirable.

KEY WORDS: Lysozyme. Albumin. Sodium citrate. Standard tear fluid (STF). Non enzymatic cleaner

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RESUMEN

En las lágrimas están presentes lisozima y albúmina, además de otros constituyentes. Todos estos componentes forman una

biopelícula en las lentes de contacto hidrofílicas y minutos después de la lente se coloca en el ojo. Estos depósitos, si no se eliminan, hacen el contacto con la lente translúcida y afectan la agudeza visual. Para eliminar los depósitos se utiliza una solución multiuso.

En el estudio, se hicieron deliberadamente depósitos de lisozima y albúmina en lentes de contacto hidrofílicas. Estos depósitos sobre los lentes de contacto fueron tratados con la solución multiuso durante 12 horas. El grado de eliminación de estos depósitos por la acción del citrato de sodio en solución multiuso se evaluó mediante la medición de la albúmina y la lisozima cuantitativamente, mediante procedimientos analíticos.

Se observó que el citrato de sodio al 0,1% podría eliminar la lisozima y albúmina de manera eficiente. La Albúmina se depositó más en comparación con la lisozima y las lentes de contacto hidrofílicas no iónicas son menos proclives al depósito que las iónicas. Cualquier aumento posterior de citrato de sodio es indeseable.

PALABRAS CLAVE: Lisocima. Albúmina. Citrato sódico. Fluído standard de lágrimas (STF). Limpiador no enzimático.

INTRODUCTION

In the eye besides tear, other constituents are also present like proteins, lysozyme, albumin and salts including calcium. All these form a lipoprotein surface film on the hydrophilic contact lenses and other contaminants are adsorbed on this film further. The contaminants may be the environmental pollutants such as nicotine, cosmetic ingredients, finger dirt, chemical vapors, water impurities and preservatives/active ingredient from ophthalmic products[1].

Certain other lipid secretions from the eye glands (meibomian glands) can also bind to the lens surface, forming a lipoprotein film that is very difficult to remove. All such deposits if not removed then may cause discomfort and impair visual acuity. Microorganisms may further build up on these deposits and the situation further worsens. To remove such deposits, the lenses are to be treated every day with multipurpose solution (MPS) containing a deproteiniser. The deposits not only cause discomfort but also increase the risk of infection causing giant papillary conjunctivitis (GPC)[2].

The enzyme cleaners provide effective cleaning but leave around 25% of the lens surface area still coated. One of the functions of multipurpose solution (MPS) is to remove lens deposits when lenses are soaked in the solution overnight. In this way, it extends the useful life of the lens and keeps the lens free from deposits and thereby provides clear vision, comfort and maintain normal eye health[3].

During day time the lenses which are previously rinsed with MPS before being worn and during night, the lenses when not in use are soaked in MPS for 7-8 hours. Lens can be worn continuously for 7-8 hours in a day, after this again rinsed and soaked in MPS for 7-8 hours before being worn again[4].

MATERIAL AND METHODS

1. Materials

Polyhexanide hydrochloride (PHMB.HCl) was procured from Avecia Biocides, Manchester, U.K. Sodium citrate was obtained from Merck, Mumbai, India and Lysozyme from SRL, Mumbai, India. Albumin was obtained from E Merck, Mumbai, India. FDA group I (Netrafilcon A) hydrophilic contact lenses were used. All other materials were used as received.

2. Preparation of standard tear fluid (STF) containing deposition constituents

STF of pH 7.4 was prepared (Table I).

Table I: Standard tear fluid (STF) pH 7.4

S. No.	Ingredient	Concentration (%)
1	Boric acid	0.2
2	Sodium tetraborate	0.02
3	Sodium chloride	0.8
4	Purified water q. s. to	100.0 ml

Albumin and lysozyme (Table II) were added into isotonic STF of pH 7.4 by shaking the flask until a clear solution was obtained.

The volume was made and pH was adjusted up to 7.4 using pHmeter.

Table II: Artificial tear fluid containing deposition constituents

S. No.	Ingredient	Concentration
1	Albumin	20µg/ml
2	Lysozyme	20µg/ml
3	STF (pH 7.4) q. s. to	100 ml

3. Selection of hydrophilic contact lenses and MPS

Group I (Netrafilcon A) hydrophilic contact lenses were used for the study. For one MPS, six contact lenses were used. The total contact lenses were 42.

Container used: Transparent vial of 10.0 ml capacity were used for the study.

MPS tested: Seven selected MPS were subjected to deposition studies i.e. MPS-2, MPS-6, MPS-7, MPS-8, MPS-9, MPS-10 and MPS-11

4. Method

In the deposition studies, two main constituents, which are generally deposited on the surface of the contact lens, are lysozyme and albumin. In the present study the removal of lysozyme and albumin from the deposited hydrophilic contact lenses (CL's) by the action of MPS was studied in order to assess the formulation. Eleven preparations of MPS were prepared and coded (Table III).

Table III: Formulae of different multipurpose solutions (MPS) concentration of ingredients in formula code (in %)

S. No.	Ingredients	MPS-1	MPS-2	MPS-3	MPS-4	MPS-5	MPS-6	MPS-7	MPS-8	MPS-9	MPS-10	MPS-11
1	PHMB.HCl	0.0001	0.0002	0.0003	0.0004	0.0005	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002
2	Sodium Citrate	0.1	0.1	0.1	0.1	0.1	0.05	0.07	0.15	0.17	0.2	0.3
3	Isotonic STF (pH 7.4) q.s.	100	100	100	100	100	100	100	100	100	100	100

All these preparations contain polyhexanide hydrochloride (PMHB.HCl) as the drug and sodium citrate as the deproteiniser and the concentration of the drug varied from 0.0002 to 0.0005% and the concentration of sodium citrate (deproteiniser) varied from 0.05 to 0.30%. In the deposition studies, lysozyme and albumin were added into an isotonic simulated tear fluid (STF) of pH 7.4 in known concentration and the hydrophilic contact lenses were soaked in it for 24 hours at 37°C in order to make coatings of lysozyme and albumin on them i.e. deposits were made on the lenses deliberately and according to the composition as given in tables (Table II and Table III). These lenses were then soaked in MPS for 12 hours and the lysozyme and albumin were estimated in order to assess the deposit removing capacity of MPS [5].

In 42 vials, the artificial tear fluid containing lysozyme and albumin were added (5.0 ml in each vial). In each of 42 vials the hydrophilic contact lens was placed. All the vials were stopper with their respective caps and placed in biological shaker at 37°C. These were shaken for 24 hours. After 24 hours, the lenses were removed with the help of contact lens lifter and placed in separate vial containing 5.0 ml of MPS.

For one MPS, six vials were used and in each vial one lens was placed. These vials were left for 12 hours at room temperature i.e. 25°C. After this these vials were shaken for 5 minutes and lenses were removed. The treated MPS were analyzed for the deposition of lysozyme and albumin, removed from the lenses, by the following methods.

4.1. Estimation of lysozyme

It was determined as per the method of Hu et al[7]. Different concentrations of lysozyme were prepared in STF of pH 7.4 i.e. 2.0 µg/ml to 20.0 µg/ml. The absorbance of the solutions were determined at λ_{max} 280 nm using UV spectrophotometer. From the readings (Table IV), a standard plot was prepared.

Table IV: Absorbance of different concentration of lysozyme at λ_{max} 280 nm

S. No.	Concentration (µg/ml)	Absorbance	SD±
1	2	0.112	0.002
2	4	0.120	0.001
3	6	0.128	0.002
4	8	0.136	0.003
5	10	0.144	0.001
6	12	0.152	0.002
7	14	0.160	0.002
8	16	0.168	0.002
9	18	0.176	0.001
10	20	0.184	0.002

n = 6, results are the mean of 6 readings.

In deposition studies, the treated MPS preparations were taken and the absorbance were determined at 280 nm. The amount of lysozyme was determined by using the standard plot as per the method. From the readings, a bar chart was plotted for showing the effect of MPS on removal of lysozyme from hydrophilic contact lenses (CL's)[6].

4.2. Estimation of albumin

Albumin was determined as per modified Lowry method[7]. A stock solution of albumin 100 µg/ml was prepared in STF of pH 7.4. From this stock solution, an appropriate volume was transferred into a 10.0 ml capacity volumetric flask. To this 1.0 ml of biuret reagent and 1.0 ml of phenol were added. After 5 minutes, volume was adjusted up to 10.0 ml. In this manner, all reaction mixture were prepared containing different concentrations of albumin i.e. 3.88 µg/ml to 42.68 µg/ml. Albumin gave intense red color in the presence of biuret agent and phenol. The absorbance of these solutions was measured spectrophotometrically at 700 nm (Table VI).

Table VI: Absorbance of different concentration of albumin at λ_{max} 700 nm

S. No.	Concentration (µg/ml)	Absorbance	SD±
1	3.88	0.105	0.002
2	7.76	0.113	0.003
3	11.64	0.121	0.003
4	15.52	0.129	0.003
5	19.4	0.137	0.002
6	23.28	0.145	0.001
7	27.16	0.153	0.002
8	31.04	0.161	0.001
9	34.92	0.169	0.003
10	38.8	0.177	0.002
11	42.68	0.185	0.002

From the absorbance and concentration value, a standard plot was drawn. In deposition studies, the treated MPS was taken and to this the biuret agent and phenol were added as the method given above. The absorbance was determined and the amount of albumin was calculated using the standard plot.

From the readings a bar chart was plotted, showing the effect of MPS solution on removal of albumin (Table VII).

Table VII: Amount of albumin removed (in µg) by multipurpose solutions (i.e. Albumin removed from contact lenses)

S. No.	Multipurpose solution	Lens No. 1	2	3	4	5	6	Average amount (µg)	SD±
1	MPS-2	16.88	16.85	17.04	17.08	17.25	17.15	17.04167	0.1546
2	MPS-6	8.2	8.55	8.15	8.5	8.13	8.1	8.271667	0.1995
3	MPS-7	10.58	11.12	11.35	10.68	11.03	11.02	10.96333	0.286
4	MPS-8	17.11	17.23	17.87	17.88	17.79	17.92	17.63333	0.3634
5	MPS-9	17.5	17.62	17.65	17.55	17.43	17.5	17.54167	0.0823
6	MPS-10	17.8	17.85	17.9	17.83	17.8	17.79	17.82833	0.0417
7	MPS-11	17.95	17.96	18.01	17.91	17.89	17.96	17.94667	0.0423

RESULTS:

Seven MPS coded as MPS-2, MPS-6, MPS-7, MPS-8, MPS-9, MPS-10 and MPS-11 were tested for their deposits removal capacity and efficiency upon treatment of the deposits laden hydrophilic contact lenses (Table III).

In the study, the deposits of lysozyme and albumin were made on the contact lenses deliberately by soaking them in STF of pH 7.4 containing the above constituents for 24 hours at 37 ± 0.5°C. Hydrophilic contact lenses of group I (Netrafilcon A) were used. The deposits laden contact lenses were then treated with MPS for 12 hours. Sodium citrate (deproteiniser) present in MPS in concentration of 0.1%, 0.05%, 0.07%, 0.15%, 0.17%, 0.20% and 0.30% in MPS-2, MPS-6, MPS-7, MPS-8, MPS-9, MPS-10 and MPS-11 respectively removed lysozyme. The average amount of lysozyme in µg removed per lens by MPS-2, MPS-6, MPS-7, MPS-8, MPS-9, MPS-10 and MPS-11 were 7.52, 3.63, 5.31, 7.48, 7.87, 8.06 and 8.21 respectively (Table V).

Table V: Amount of lysozyme removed (in µg) by multipurpose solution from contact lenses

S. No.	Multipurpose solution	Lens No. 1	2	3	4	5	6	Average amount (µg)	SD±
1	MPS-2	7.13	7.71	7.75	7.25	7.69	7.6	7.52	0.2643
2	MPS-6	3.69	3.45	3.4	3.6	3.8	3.85	3.63	0.1828
3	MPS-7	5.01	5.2	5.31	5.45	5.3	5.6	5.31	0.2029
4	MPS-8	7.25	7.81	7.68	7.4	7.3	7.45	7.48	0.2198
5	MPS-9	7.8	7.9	7.98	7.86	7.69	7.97	7.87	0.1098
6	MPS-10	8.1	8.13	7.99	7.98	8.01	8.12	8.06	0.0689
7	MPS-11	8.2	8.16	8.22	8.31	8.27	8.1	8.21	0.0754

Similarly average amount of albumin removed per lens in µg by MPS-2, MPS-6, MPS-7, MPS-8, MPS-9, MPS-10 and MPS-11 were 17.04, 8.27, 10.96, 17.63, 17.54, 17.83 and 17.95 respectively (Table VII).

The removal occurred on the surfaces of non-ionic hydrophilic contact lenses, which are FDA approved, group I and group II types[8]. The albumin was deposited more than lysozyme and the capability of the MPS with sodium citrate used as deproteiniser was more towards lysozyme.

The lysozyme could be removed easily than albumin by the MPS for hydrophilic contact lenses.

0.1% of sodium citrate in MPS for hydrophilic contact lenses is upto the mark. Sodium citrate 0.1% can be used as deproteiniser for removal of lysozyme and albumin present on the surface of hydrophilic contact lenses.

DISCUSSION:

One reason for removing contact lens deposits is to extend the useful life of the contact lens. The more important reasons for cleaning hydrophilic contact lenses are to maintain clear vision, good comfort, and most importantly normal eye health. Undesirable organic substances within the tear film layer, such as lipids, mucoproteins, albumin, immunoglobulin, glycoproteins, mucin and lysozyme combine with inorganic compounds, bacteria and microorganisms to form a complex biofilm deposit on contact lens surface within minutes of placing the lens on the eye. These deposits continue to build on the contact lens surface with successive wearing period, eventually causing discomfort from mechanical irritation of the ocular tissues, as well as blurred vision as the optical quality of the contact lens surface degrades. This biofilm can also act as an antigenic stimulus causing allergic lid reactions such as giant papillary conjunctivitis (GPC).

GPC causes blurred vision, reduced wearing time, redness, itching, stinging, ocular discomfort and mucous discharge. GPC used to be a frequent occurrence with hydrophilic contact lenses but with the advent of multipurpose solution with non enzymatic cleaners like citrate, tris, hydranateTM the incidence of GPC in hydrophilic contact lens wearer has decreased considerably.

Sodium citrate is a non enzymatic deproteiniser used in MPS. It's a trivalent anionic molecule with chelating properties. It is effective in removing protein, lipids and polysaccharide deposits from contact lenses surface and breaks calcium bridges which link protein deposits to each other and to the lens. The cleaning activity and no ocular toxicity promote longer lens life. Citrate is therefore used in MPS for the overnight storage of the lens and for rinsing and soaking of the contact lenses. Citrate is a non enzymatic deproteiniser in the MPS.

Tear proteins such as lysozyme and albumin are large multivalent molecules containing both positive and negative local areas of charge. The positively charged sites on protein molecule can form ionic bonds with the negatively charged surface of the ionic contact lens and thereby binding proteins to the surface. One protein layer on to the other therefore builds up within no time, this ionic building is strong type hence to remove such bindings one has to store the lenses overnight and also to clean, rinse the lens by mechanically rubbing with MPS. This cause the removal of deposit coupled by weekly cleaning using an enzymatic cleaner as well.

In the experiment, the non ionic lenses are used because here the binding is not that strong and non enzymatic cleaner which are less allergy prone like sodium citrate has been used. There is no strong binding hence no need of enzymatic cleaner like papain which induce allergy. Hence sodium citrate could solve the purpose and is therefore used as deproteiniser. Binding occurs but not to that extent as in ionic type and this binding is broken by the compound like citrate[8].

In order to ascertain the efficiency of MPS preparation towards the removal of deposits from the contact lenses, a deposition study was performed. In the study, lysozyme and albumin were measured quantitatively i.e. removal of these deposits from the surface of the lens has been studied. Seven MPS preparations i.e. MPS-2, MPS-6, MPS-7, MPS-8, MPS-9, MPS-10 and MPS-11 were tested for their deposits removal efficiency upon treatment of the used lenses by them. These preparations differ in the sodium citrate concentration- the deproteiniser. The concentration of sodium citrate varied from 0.05% to 0.3% in these formulations.

In the study, the deposits of lysozyme and albumin were made on the contact lenses deliberately by soaking them in STF of 7.4 containing these components for 24 hours at 37±0.5°C. Group I (Netrafilcon A) were used for the reasons given above. These lenses are non ionic type. These deposits laden contact lenses were then treated with MPS preparation for 12 hours. During the treatment period, the deposits of lysozyme and albumin were removed by the action of sodium citrate present in the MPS. The extent of removal of these deposits was assessed by measuring albumin and lysozyme quantitatively by using standard analytical procedures discussed in the section. It was observed that the preparation containing less amount of sodium citrate i.e. 0.05% in MPS-6 removed less amount of lysozyme and albumin.

It was further ofserved that(the preparation containing more amount of sodium citrate i.e. MPS-7 and MPS-2 (0.07% of sodium citrate MPS-7 and 0.1% in MPS-2) removeÐ these deposits in increasing orders. However, further increase of sodium citrate i.e. beyond 0.1% in the preparation of MPS-8, MPS-9, MPS-10 and MPS-11 remove the deposits, slightly more as sompared to MPS-12ˆbut not significantly. The result of MPS-2 preparation was comparable with other preparations i.e. MPS-8, MPS-9, MPS-10 and MPS-11. This is due to the fact that entire deposits were removed by MPS-2 preparation containing 0.1% of sodium citrate; therefore further increase in the sodium citrate concentration was not desirable i.e. the concentration of sodium citrate in the preparation MPS-8, MPS-9, MPS-10 and MPS-11 were in excess. Hence MPS-2 is an optimized preparation as far as quantity of sodium citrate is concerned.

It was further observed that deposits due to albumin were more as compared to lysozyme. The solution with 0.1% of sodium citrate could remove lysozyme more efficiently than albumin. Non ionic contact lenses - group I (Netrafilcon A) were used because these lenses actually discourage binding within the polymeric network of hydrophilic contact lenses. The binding is weak enough that non enzymatic cleaner like sodium citrate can break it easily.

From the deposition studies it was concluded that MPS-2, the multipurpose solution preparation, gave better results in the removal of deposits from the surface of the hydrophilic contact lens as compared to the other prepa

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Comment of the reviewer Prof. Pilar Muñiz Rodriguez. PhD. Professor of Biochemistry and Molecular Biology, Faculty of Science. University of Burgos. España

In this paper, the authors present the results of a study on the effect of different concentrations of sodium citrate present in a multipurpose solution commonly used for cleaning hydrophilic contact lenses. In the study, the authors observed that a concentration of 0.1% of sodium citrate was optimal in the removal of lisozyme and albumin.

Although the study was in some ways limited, the results obtained are interesting because of their usefulness to both the prolongation of the life of the contact lenses as well as on the eye health.

Comment of the reviewer Victoria Valls Bellver PhD. Biochemistry. Department of Pediatry and Ginecology. University of Valencia. España

The authors describe a method for treating lypophilic contact lenses using the multipurpose solutions (MPS). MPS are the

solutions most prescribed with all lenses because of their benefits of convenience, simplicity, and disinfections properties.

It is know that among the organic substances that could deposit on contact lens, are the albumin or lysozyme that combination with bacteria or microorganism to form a biofilm on contact lens. In this work, the authors compare MRP with different concentrations of sodium citrate, showing the best results to the concentration of 0.1% where the release of albumin and lisozyme from the lens was higher.

The applicability of this study could to help prevent complications associated to eye allergies.

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