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OPTIMIZATION OF CONCENTRATION OF POLYHEXANIDE HYDROCHLORIDE IN MULTIPURPOSE SOLUTION.

A. Arora, A.Ali*, M.T.Zzaman[1], S.Chauhan, V.Handa

**KIET School of Pharmacy. Ghaziabad Meerut Road. Ghaziabad.
*Jamia Hamdard, Faculty of Pharmacy, Dept of Pharmaceutics. Hamdard Nagar. New Delhi
India.**

alkamailin @ yahoo.co.in

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Comment of the reviewer Comment of the reviewer Erhan Süleymanoglu PhD. **G.U.E.F., Department of Pharmaceutical Chemistry, Gazi University. Gazi Mahallesi, Ankara. Turkey.**

Comment of the reviewer Maria Angeles Mantecón Vallejo PhD. **Microbiología. Complejo Asistencial Universitario de Burgos. España**

ABSTRACT

There are a number of foreign pathogenic microorganisms like viruses, bacteria, yeast, fungi and protozoa which can inadvertently be introduced into eye via contact lens and hence disinfection is a vital part. Multifunctional solutions are generally intended to combine the action of cleaning disinfecting, rinsing lubricating deproteinising and soaking in one single product.

In order to optimization of concentration of Polyhexanide hydrochloride in multipurpose solution some microbiological studies were performed by taking five microorganisms which are more prevalent in the infected eye condition. The nutrient agar and sabourad's agar media were used for bacteria and yeast mould respectively. The media were prepared as per I.P and sterilised by autoclaving and poured into Petri plates. The media when cooled to 42°C, 0.5 ml of the culture was added.

The optimum concentration of Polyhexanide hydrochloride is 0.0002% which is an effective concentration against five microorganisms that are most prevalent in the infected eye condition.

It was concluded that the multipurpose solution containing 2.0 g/ml of polyhexanide were found to be better in terms of antimicrobial activity

Keywords: Zone of inhibition. Disinfection. Ocular pathogens.

RESUMEN: OPTIMIZACIÓN DE LA CONCENTRACIÓN DE POLIHEXANIDE CLORHIDRATO EN SOLUCIÓN POLIVALENTE.

Hay un número de microorganismos extraños patógenos como virus, bacterias, levaduras, hongos y protozoos, que pueden introducir en el ojo inadvertidamente, a través de lentes de contacto y por lo tanto, la desinfección de las lentes es vital. Las soluciones multifuncionales, generalmente destinadas a combinar la acción de limpieza, desinfección, aclarado, lubrificación y deproteinización pueden combinarse en en un solo producto.

Para la optimización de la concentración de clorhidrato de Polyhexanide en solución multiuso se realizaron varios estudios microbiológicos, mediante el empleo de los cinco microorganismos que son más frecuentes en la infección ocular. Para ello se utilizaron medios de cultivos con agar nutritivo y Sabourad para las bacterias y levaduras respectivamente. Los medios de cultivo fueron preparados por IP, esterilizados en autoclave y vertidos en placas de Petri. Una vez enfriados a 42° C, se añadieron 0,5 ml de cultivo.

La concentración óptima de clorhidrato de Polyhexanida es de 0.0002%, que es efectiva contra los cinco microorganismos más prevalentes en la infección ocular.

Se concluyó que la solución multiuso que contiene 2,0 g/ml de polyhexanida resultó ser la mejor en términos de actividad antimicrobiana.

Palabras clave: Zona de inhibicion. Disinfeccion. Patógenos oculares.

INTRODUCTION

Contact lens care systems have two major functions i.e. cleaning and disinfection. Cleaning may consists of daily cleaning with a separate surfactant cleaner at the end of the wearing period or simple mechanical rubbing of the lens on the palm of the hand with an all in one solution. The drug in the contact lens solution should have surface active characteristics as well as the antibacterial, antifungal and antiprotozoal properties. The most serious complication of the contact lens wear is the sight threatening ocular infection.

A number of potentially pathogenic microorganisms normally exist in the eye as normal ocular flora without causing disease. However under the right condition opportunistic microorganisms can give rise to serious ocular infection. There are a number of foreign pathogenic microorganisms like viruses, bacteria, yeast, fungi and protozoa which can inadvertently be introduced into eye via contact lens and hence disinfection is a vital part. The two most widely used methods of contact lens disinfection have been heat and cold chemical disinfection. Heat disinfection requires a heating unit, a heat resistant contact lens case and electrical power which may not be available always. Heat is rarely used therefore cold chemical disinfection is widely used method [1-2].

1.1 Parameters for the selection of the Drug

Following parameters should be considered for the selection of the drug for multipurpose all in one solution for hydrophilic contact lenses

(1) The drug should have surface activity i.e. cleaning action.

(2) The drug should have antibacterial antifungal antiprotozoal and antiacanthamoeba activity and should be potent.

(3) The drug should be non irritant to the eyes.

(4) It should be non toxic to the ocular tissues.

(5) The drug should not affect non ionic and ionic type of hydrophilic contact lenses and gas permeable contact lenses.

(6) The drug should not bind itself with tear film like mucin, lipids mucoproteins, albumin, immunoglobulins, glycoproteins and lysozyme.

In the present research work Polyhexanide was selected in multipurpose solution due to the following reasons[3-6].

1.- Polyhexanide hydrchloride at a concentration of 0.0001% is an effective chemical disinfecting agent against the microorganisms typically found on contact lenses.

2.- There has been no report of corneal toxicity or allergic reaction till date.

3.- It is currently used in an all in one contact lens solution for soft and rigid gas permeable contact lenses. The nine brands are available in the market including COMPLETE manufactured by (Allergan). RENU manufactured by (Baush& Lomb) and MULTISOL manufactured by (Gaymed labs)

4.- Polyhexanide has a faster killing rate than other antimicrobial drugs like Polyquad for *Serratia Marcescens*, *Pseudomonas aeruginosa* and *Candida albicans*.

Considering the above facts it was considered to develop multipurpose all in one solution for hydrophilic contact lenses using Polyhexanide hydrochloride as main constituent.

MATERIALS AND METHODS

Polyhexanide hydrochloride was procured from excel industries New Delhi India. The buffering and isotonic agent sodium tetraborate, boric acid and sodium chloride were obtained from Merck Mumbai India. All the reagents used were of Analytical grade.

The cup and plate method as per I.P. was used. The microbiological studies were performed by taking five microorganisms which are more prevalent in the infected eye condition. The nutrient agar and sabourad's agar media were used for bacteria and yeast mould respectively. The media were prepared as per I.P and sterillised by autoclaving and poured into Petri plates. The media when cooled to 42 C, 0.5 ml of the culture was added.

In the seeded solidified media plates the cups were cut in aseptic condition. The cups were filled with multipurpose solution and zone of inhibition was read and then compared. The optimum concentration of Polyhexanide hydrochloride is 0.0002% which is an effective concentration against five microorganisms that are most prevalent in the infected eye condition.

The following microorganisms were used for the study

Gram positive bacteria	***Staphylococcus aureus*** **(cfu $7x10^7$/ml) Strain no: ATCC 6538**
Gram negative bacteria	***Pseudomonas aeruginosa*** **(cfu $6x10^7$/ml) Strain no: ATCC 15442** ***Serratia marcescens*** **(cfu $5x10^7$/ml) Strain no: WHO**
Yeast	***Candida albicans*****(cfu $4x10^7$/ml) Strain no ATCC 10231**
Mould	***Aspergillus fumigatus*** **(cfu $6x10^7$/ml) Strain no ATCC 10894**

All the microorganisms were procured from IMTECH Chandigarh Punjab India.[7-10].

RESULTS

The results are sumarized in the following tables 1-4, and figures 1-4.

Table 1.- Polyhexanide hydrochloride concentration in multipurpose solution

Formulation	Polyhexamide Hcl in isotonic simulated tear fluid of pH 7.4 as per U.S.P (STF pH 7.4)
MPS-1	0.0001%
MPS-2	0.0002%
MPS-3	0.0003%
MPS-4	0.0004%
MPS-5	0.0005%
MPS-6	0.0002%
MPS-7	0.0002%
MPS-8	0.0002%
MPS-9	0.0002%
MPS-10	0.0002%
MPS-11	0.0002%

Table 2. Characterization of Multipurpose Solution (MPS)

Formula code	Appearance	pH	Assay of Polyhexanide .HCl (µg/ml)	Viscosity (cps)	Surface tension (dynes/cm)	Refractive Index	Test for Sterlity
MPS-1	Clear colourless liquid	7.42	1.04	1.20	42.4	1.315	Passed
MPS-2	Clear colourless liquid	7.40	2.02	1.22	42.3	1.316	Passed
MPS-3	Clear colourless liquid	7.41	3.00	1.19	42.3	1.317	Passed
MPS-4	Clear colourless liquid	7.45	4.12	1.20	42.2	1.316	Passed
MPS-5	Clear colourless liquid	7.44	5.04	1.20	42.0	1.317	Passed
MPS-6	Clear colourless liquid	7.45	2.05	1.22	42.1	1.315	Passed
MPS-7	Clear colourless liquid	7.40	2.02	1.19	42.1	1.317	Passed
MPS-8	Clear colourless liquid	7.42	2.04	1.19	42.3	1.315	Passed
MPS-9	Clear colourless liquid	7.43	2.05	1.20	41.9	1.316	Passed
MPS-10	Clear colourless liquid	7.44	2.01	1.21	42.0	1.315	Passed
MPS-11	Clear colourless liquid	7.43	2.05	1.22	42.2	1.315	Passed

Table 3. Microbiological Evaluation of MPSs by Cup Plate Method

MPS code No	Zone of inhibition (mm) against				
	S.aureus	*P.aeruginosa*	*S.marcescence*	*C.albicans*	*A.fumigatus*
MPS-1	19.9	20.1	19.8	18.0	17.4
MPS-2	25.4	24.9	25.2	22.0	22.2
MPS-3	25.7	25.5	25.8	22.8	22.7
MPS-4	25.9	25.7	26.2	23.2	22.9
MPS-5	26.1	25.8	26.4	23.5	23.2
MPS-6	26.4	24.8	25.4	22.2	21.9
MPS-7	25.9	24.9	25.3	22.2	21.9
MPS-8	25.7	24.9	25.4	22.4	22.4
MPS-9	25.0	25.0	25.2	22.3	22.3
MPS-10	26.0	24.8	25.2	22..0	22.4
MPS-11	25.9	24.8	25.5	22.9	22.1

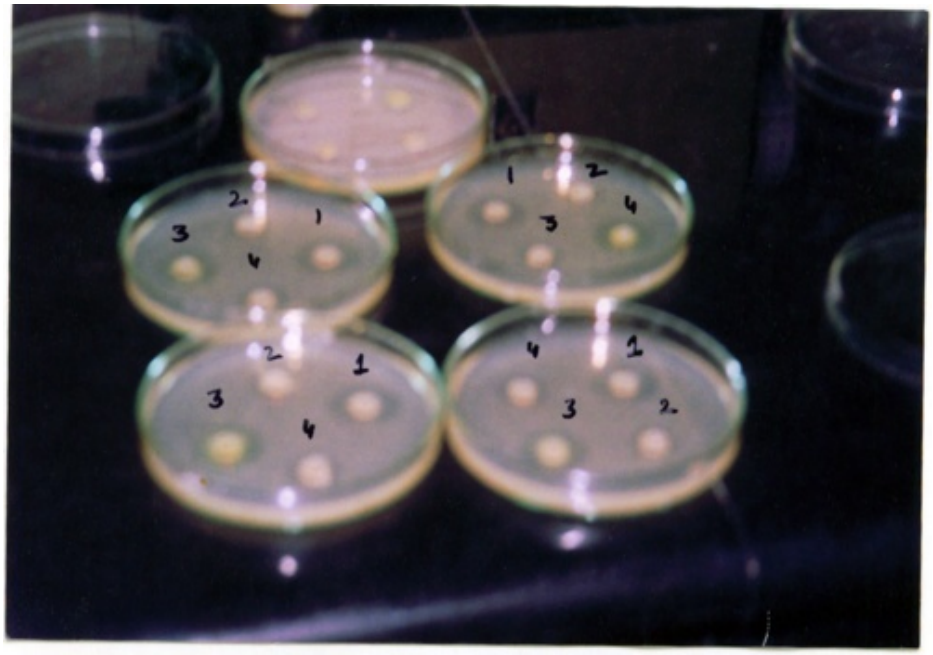

Figure 1.- Zone of Inhibition with *S.aureus*

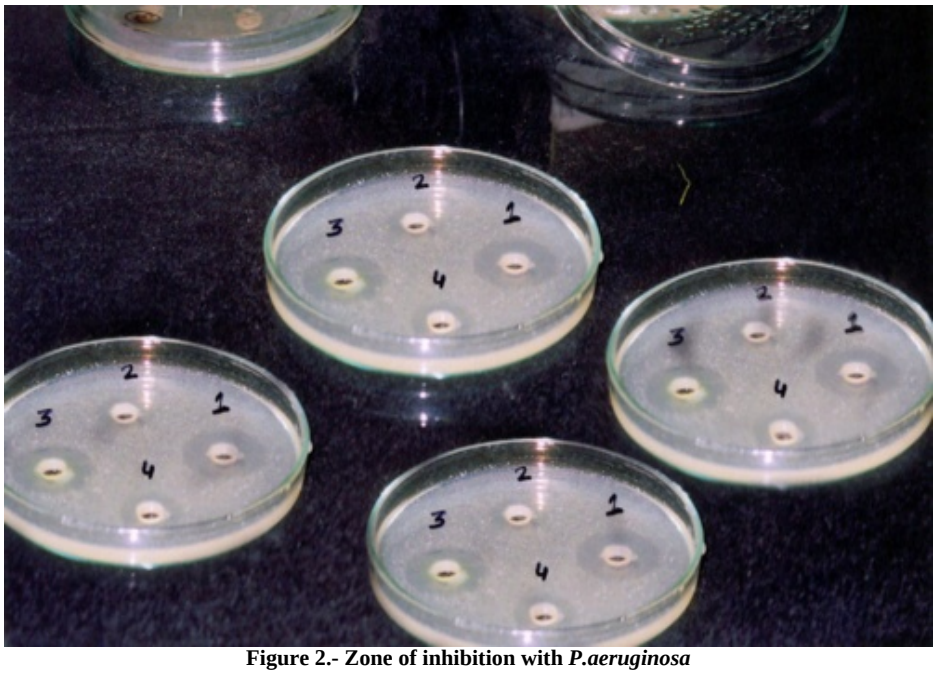

Figure 2.- Zone of inhibition with *P.aeruginosa*

Table 4. Microbiological Evaluation of MPS and Marketed Multipurpose solution

Formulation	Zone of Inhibition (mm) against				
	S.aureus	*P.aeruginosa*	*S.marcescence*	*C.albicans*	*A.fumigatus*
MPS-2 (Cup No 1)	25.2	25.0	25.1	22.2	22.0
MPS-2 Placebo (Cup No. 2)	Very slight due to edetate	Very slight due to edetate	Very slight due to edetate	–	–
Marketed-1 Multipurpose solution (**COMPELETE**) (MR-1) (Cup No. 3)	23.7	23.8	24.8	21.4	19.0
Marketed-2 Multipurpose solution (**RENU**) (MR-2) (Cup No.4)	23.2	23.2	24.0	20.8	19.9

N = 4 result are the mean of four readings. Cup No.2 = Control

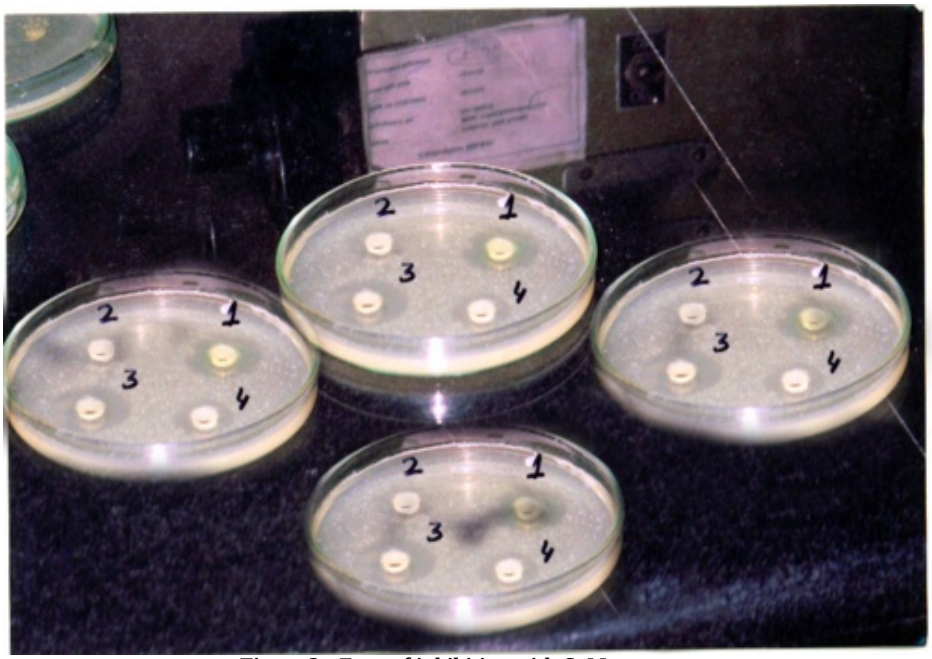

Figure 3.- Zone of inhibition with *S. Marcescens*

Figure 4.- Zone of inhibition with *C.albicans*

On the basis of the above and over all observation and findings it was concluded that the multipurpose solution containing 2.0 g/ml of polyhexanide were found to be better in terms of antimicrobial activity i.e. preparation code No MPS-2,MPS-6, MPS-7, MPS-8, MPS-9, MPS-10 and MPS-11.

DISCUSSION

Multifunctional solutions are generally intended to combine the action of cleaning disinfecting, rinsing lubricating deproteinising and soaking in one single product. The rationale behind the manufacture of such solution is that some wearers will not carry out correct lens hygiene procedures and may omit one or the other steps due to the confusion of the multiplicity and expense of the solution.

Most contact lens practitioners have come across patient who omits one or more steps due to forgetfulness, carelessness or due to cost factor because for six steps, six different solutions are needed. Due to this non compliance many risks can occur. These risks include acute and chronic red eye, corneal abrasions, giant papillary conjunctivitis (GPC), sterile infiltrates, corneal scarring and neovasculization. However the most serious and severe complication associated with contact lens wear is infectious keratitis[11-12].

The infective keratitis is caused by invasion of the offending pathogens into the cornea. If not properly treated the infection can progress quickly destroying valuable corneal storma and possibly lead to corneal perforations, scarring and permanent loss of vision. Even with prompt and aggressive treatment with appropriate topical antibiotic, loss of vision may occur. Therefore steps must be taken to minimize the risk of infective keratitis in contact lens wearers[1, 3].

Multipurpose and multifunctional solutions are designed in such a way that they fulfill all six steps in one single step so that the patient compliance is increased. The six steps cumbersome method is now one step method which is economically viable. The minimum chances of microbial contamination are also an important factor in using multipurpose solution and lens care products. Three main issues are critical in developing contact lens care products i.e. efficacy, safety and convenience

As might be expected, the combination of different lens hygiene function into multifunctional solution has elicited discussion about a possible compromise of efficacy in these products. For example, the relatively high viscosity required for a mechanical buffer action is contrary to the low viscosity required for diffusion of surface contaminants into the storage solution. Further, solution viscosity of any degree would appear to retard bactericidal activity as shown by the proper performance of all combination wetting and soaking solution tested by Knool in 1974 compared to soaking solution alone.

Whilst acknowledging some compromise the practitioner may feel that certain patients e.g. through lack of mental ability, application or responsibility or simply because of occupational factors such as large amount of traveling or when on holiday, should be advised to use multipurpose solution

During the early stage in the development of hydrophilic lenses it was thought that the hydrophilic nature of the surface would obviate the need for most solutions since wetting agents were obviously unnecessary and for the same reason it was thought that cleaning would also be unnecessary.

However, as experience has been gained over the last few years the complexities of dealing with new materials for use in contact with the eye has become increasingly apparent. Further, whilst the large majority lenses are still made from a single material (PMMA), the hydrophilic lenses are made from many materials (HEMA), including differing additives, and with widely varying

physical and physiological properties

The microbiological studies of multipurpose solution (MPS-1 to MPS-11) were performed using cup plate techniques against Gram positive bacteria (*S. aureus* cfu 7x10 7/ml ATCC 6538) Gram negative bacteria (*P. aeruginosa* cfu 6x107/ml, ATCC 15442 and (*S. marcescens* cfu 5x107/ml WHO) yeast (*C. albicans*, cfu 4x10 7/ml, ATCC 10231) and mould (*A. fumigatus*, cfu 6x107/ml ATCC 10894)

The experiment was repeated six times and average zones of inhibition were calculated. In case of MPS-1lesser zone of inhibition value was obtained for all types of microorganisms as compared to other preparation MPS-2 to MPS-11. Significantly. The values of zones of inhibition in preparation MPS-2 were found to be on the higher side for all micro organisms used. In case of preparation MPS-3, MPS-4, MPS-5 the zones of inhibition values were found to be slightly in increasing order but not significantly. The antimicrobial activity of the preparation MPS-2 was almost comparable with the preparation MPS-3, MPS-4 and MPS-5.

In other preparations like MPS-6,MPS-7,MPS-8,MPS-9,MPS-10and MPS-11, the antimicrobial activity was almost similar to the preparation MPS-2 and this might be due to the presence of similar concentration of Polyhexanide hydrochloride i.e. 2.0 g/ml. On the basis of these observations it was concluded that concentration of 2.0 g/ml of Polyhexanide in multipurpose solution gave desired and satisfactory antimicrobial activity and unnecessarily higher concentration of Polyhexanide hydrochloride i.e. more than 2.0 g/ml can be avoided. As per the solution containing Polyhexanide hydrochloride 5.0 g/ml becomes ocular irritant.

The antimicrobial activity of MPS-2, MPS-2 placebo (control) and other two multipurpose solutions were determined in the same manner as above. It was found that the antimicrobial activity against all five types of microorganisms was higher in case of MPS 2 preparation as compared to the other two marketed preparation of multipurpose solution called COMPELETE and RENU as it was evident from the zones of inhibitions and bar diagram[14]

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CORRESPONDENCE:
Dr. A. Arora
B-264 Derawal Nagar, opp.
Model Town-II,
Delhi-110009, India.
Email: alkamailin @ yahoo.co.in

Comment of the reviewer Erhan Süleymanoglu. G.U.E.F., Department of Pharmaceutical Chemistry, Gazi University. Gazi Mahallesi, Ankara. Turkey.

The present work represents an attempt to assess the effects of newly suggested polyhexanide HCl asa multipurpose solution for hydrophilic contact lenses. The study highlighted differences regarding the microbiological profiles of this solution against selected 5 bacterial species. By carefully estimating the zones of inhibition, the authors deduce the desired and satisfactory polyhexanide antimicrobial activity in the multipurpose solution.

The data would afterwards serve for a beter understanding of the views and habits, the emphasis being put on hygiene and complience issues of contact lens wearers.

Comment of the reviewer Maria Angeles Mantecón Vallejo PhD. Microbiología. Complejo Asistencial Universitario de Burgos. España.

Eye infections associated with contact lenses, are an increasingly important disease due to the widespread of contact lenses and, above all, for the complications arising from it. The cause is usually the inappropriate use of lenses, inadequate hygiene or maintenance problems of the solution used for cleaning and disinfection. In this sense, there are developed solutions that meet the tasks of cleaning and disinfection in one step. Therefore it is important that the multipurpose solution meets the right components and the optimal concentrations to fulfill its role antiseptic and clean and not damage or irritate the eye tissues.

In this paper the authors establish the optimal concentration of Polihexano, antiseptic common component of multipurpose solutions, 2 microg / ml. At this concentration is reached greater antiseptic activity against microorganisms most prevalent in this type of infection and also not damage ocular tissues.

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