RESEARCH ARTICLE

Preparation and evaluation of hand rub disinfectant

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The present work is to formulate effective hand rub disinfectant by selecting broad-spectrum antimicrobial and antifungal agents. Increased need of easy-to-use hand rub disinfectant in pharmaceutical, food processing industry, hospitals, and in clinical labs necessitated development of the best possible product. Formulations were prepared using Benzalkonium chloride, chlorhexidine gluconate as potent disinfectants along with alcohol 70%. In low concentration they were highly effective without leaving any toxic effect on the user's skin. A synergistic effect was observed when ethyl alcohol 70% was used in combination. Suitable emollient and skin conditioning agents were used to avoid possible dehydrating effect on the user's skin. Excellent volatility of the preparation was observed after use, leaving a thin antimicrobial film on the user's hand. The use of suitable excipient brought effective removal of after the use leaving a soothening effect on the skin. The aim of present work is to develop hand rub formulation which evaporates quickly after applications leaving no trace on hands as in case of hand rub gels. The present formulation was found to be effective when compared with marketed liquid hand rub.

Key words: *Alcohol, benzalkonium chloride, chlorhexidine gluconate, ethanol, hand rub disinfectant*

INTRODUCTION

The options for keeping hands clean in hospitals include soap, antimicrobial solutions, iodine and alcohol solutions and rubs.[1] Hand washing is the single most effective and cost-effective method for preventing and reducing the transmission of infections. Hand hygiene is an encompassing term that refers to degerming or decontaminating the hands. Hand hygiene can be accomplished by several methods, including hand washing with plain water and soap, hand washing with an antimicrobial soap, or using an antiseptic hand rub product.[2-5] Most antiseptic hand rub products are alcohol-based, with or without emollients or other additives, because alcohol is known to be an effective skin disinfectant. Compliance with general hand hygiene may improve when alcohol-based products are used. Because, use of alcohol-based products are less time-consuming than hand washing, and products with emollient additives may be less irritating to the hands.[6-7] Products selected for surgical hand antisepsis should meet specific criteria and be labeled specifically for surgical hand antisepsis. Such products must be fast acting, nonirritating to the skin, effective against a broad spectrum of microorganisms and compliant with Food and Drug Administration (FDA) approval processes and protocols.[3,8,9] Benzalkonium chloride and chlorhexidine gluconate were selected as potent disinfectants. The formulation was evaluated by *in vitro* methods. It was found to be effective against a wide range of pathogenic microbial flora exhibiting good Ridal Walker coefficient.[10]

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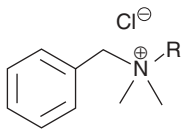
MATERIALS AND METHODS

Chlorhexidine gluconate and benzalkonium chloride were obtained from Cardiograph Corporation, Mumbai as a gift sample. Ethyl alcohol was obtained as gift sample from Healthcare pharmaceuticals, Karad. Glycerine, sorbitol, tween 80 was also obtained as gift sample from Cardiograph Corporation, Mumbai.

Benzalkonium chloride (alkyl dimethyl benzyl ammonium chloride)



$R = C_8H_{17} - C_{18}H_{37}$

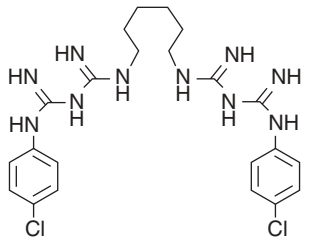
Benzalkonium chloride is an organic compound that is used as an antiseptic, disinfectant and spermicide. Benzalkonium chloride is readily soluble in water,

alcohol, and acetone, effective against Gram positive and Gram negative bacteria. When used over a longer period, there can be a selection in favor of Gram negative bacteria. It is a nitrogenous cationic surface-acting agent belonging to the quaternary ammonium group. Benzalkonium chloride is a mixture of alkylbenzyl dimethyl ammonium chlorides of various alkyl chain lengths. It is selected in the formulation because; it is one of the safest synthetic biocides known and has a long history of efficacious use. The mechanism of bactericidal/microbicidal action is thought to be due to disruption of intermolecular interactions. This can cause dissociation of cellular membrane bilayers, which compromises cellular permeability controls and induces leakage of cellular contents. Other biomolecular complexes within the bacterial cell can also undergo dissociation.[11]

Benzalkonium chloride solutions are rapidly acting anti-infective agents with a moderately long duration of action. They are active against bacteria and some viruses, fungi, and protozoa bacterial spores are considered to be resistant. Solutions may be bacteriostatic or bactericidal depending on the concentration of benzalkonium chloride. Gram positive bacteria are generally more susceptible than gram-negative. Antibacterial activity is not greatly affected by pH, but increases substantially at higher temperatures and prolonged exposure times. Applications of benzalkonium chloride are extremely wide ranging, from disinfectant formulations to microbial corrosion inhibition in the oilfield sector. It has long been deemed safe for human use, and is widely used in eyewashes, mouthwashes, spermicidal creams, and in various other cleaners, sanitizers, and disinfectants but here the formulation is specifically prepared for hand washes with low cost and high efficacy.

Formulation requires great care as Benzalkonium can be inactivated by certain organic compounds, including soap, and must not be mixed with anionic surfactants. Hard water salts can also reduce biocidal activity.

Chlorhexidine gluconate



Chlorhexidine Gluconate is a biguanidine derivative, used in concentrations of 0.5-4.0% alone or in lower concentrations in combination with other compounds, such as alcohols.[12] It can be used as a skin antiseptic and to treat inflammation of the gums. The microbicidal action is somewhat slow, but remanent. It is a cationic surfactant, similar to Quats. Chlorhexidine is efficient against both gram positive and gram negative bacteria (but Pseudomonas, Proteus and Providencia might be resistant). The effect on fungi is variable. We observed that, the bactericidal effect is enhanced by increasing the concentration of alcohol.

pH dependency - Optimum pH is 5.5-7.0 but it works in the range pH 5-8. pH values lower than 5 pH causes destruction of chlorhexidine, whereas pH values above 8.0 causes precipitation of the chlorhexidine. Low toxicity, but when used frequently through a longer period of time, a few cases of contact dermatitis and photo sensibilisation have been observed.

Alcohols

Ethyl alcohol (ethanol) and isopropyl alcohols are the most frequently used alcohols.

CH_3CH_2—OH

Alcohols kill vegetative forms of bacteria (including TB) and fungi, but have no action on spores or viruses.[13-14] Their effect depend on concentration, 70-80% alcohol inactivates HIV and Hepatitis B in 2-10 minutes. Alcohols act rapidly by precipitate proteins and solubilise lipids present in cell membranes. Ethanol is absorbent and astringent and dries out the skin.[15] Synergistic effect is seen when used together with iodine, chlorhexidine and quaternary ammonium compounds. It normally takes only 10-15 seconds for the application. If hands are drying in less than 15 seconds, it indicates that insufficient amount of alcohol disinfectant has been applied. Those solutions containing 60-95% alcohol are most effective.[15]

EXPERIMENTAL

Preparations of hand rub disinfectant

Effective hands rub disinfectant with active ingredients in Table 1 was prepared by mixing chlorhexidine gluconate, benzalkonium chloride and ethanol uniformly. Another solution of glycerine, sorbitol and tween 80 was prepared in sufficient quantity of water. Both the solutions were mixed and final volume was adjusted with distilled water.

Table 1: Composition of four different hand rub disinfectant formulations

Active Ingredient	F1	F2	F3	F4
Chlorhexidine gluconate	0.2	0.5	1	1
Benzalkonium chloride	0.2	0.5	1	1
Ethyl alcohol 70%	70	70	70	70
Glycerine	4	4	2	2
Sorbitol	2	2	1	1
Tween 80	1	2	1	2
Distill Water q.s.	100 ml	100 ml	100 ml	100 ml

*All quantities are in %W/V

Evaluations of hand rub disinfectant

In vitro antimicrobial activity testing of hand rub disinfectant. To the 5 ml aliquots of undiluted hand rub disinfectant, 0.1 ml of each bacterial culture, containing approximately 10^6 cell, was suspended individually in these tube mixed thoroughly and incubated at room temperature. After different interval of time a loop of suspension from each tube was incubated at 37°C. After 48 h, the tubes were checked for growth, indicating viability was checked for the bacterial culture. The procedure was repeated for fungal species like Aspergillus niger and Candida albicans by using Sobouraud's Broth as the nutrient Medium and their tubes were incubated at 72 h at 28°C to observe the growth or inhibition and reported in Table 2. All the apparatus used were sterilized before use.

pH

pH of 25 ml of hand rub disinfectant was determined by using pH meter (VHS Electronic MK VI) it was found to be 7.2.

Table 2: Evaluation of hand rub disinfectant formulations

Formulation	pH	Viscosity (cps)
F1	7.1	3.19
F2	7.3	3.11
F3	6.9	2.76
F4	7.0	2.73

Viscosity

Viscosity of hand rub Disinfectant was determined by using Brookfield (V-II PRO) viscometer at 100 rpm and it was found to be 3 cps.

RESULTS AND DISCUSSION

In the present study, efforts were made to prepare an effective hand rub disinfectant by using different ingredient shown in Table 1 in different percentage.

Chlorhexidine gluconate, benzalkonium chloride have selective broad spectrum activity by killing/ inactivating the test organisms E-coli, Shigella flexneri, Pseudomonas aeruginosa, Candida albican and Aspargellus niger after a killing/inactivating contact time of less than one minute. It was found that all the formulations prepared were slightly yellow in color with ethanolic odor and smooth in feel.

The pH and Viscosity of the different formulation were studied and are shown in Table 2. Amongst the four different formulations evaluated F1 and F2 were not effective against microorganisms within 60 sec [Table 3] and also had a sticky feel when used as hand rub disinfectant. While the F3 and F4 were the most effective against the microorganisms with killing/ inactivation time less than 45 sec [Table 4] but F3 suffered from miscibility problem which was due to less concentration

Table 3: *In vitro* antimicrobial activity testing of hand rub disinfectant formulation F1 and F2 on various microorganisms

Test organisms	Viability/Inhibition of organisms in undiluted form of hand rub disinfectant after a contact time in seconds (same for F1 and F2)					
	45	60	120	180	240	360
E. coli	-	n	n	n	n	n
Salmonella typhi	-	n	n	n	n	n
Staphylococcus aureus	-	n	n	n	n	n
Shigella flexner	-	n	n	n	n	n
Pseudomonas aeruginosa	-	n	n	n	n	n
Candida albicans	-	n	n	n	n	n
Aspergillus niger	-	n	n	n	n	n

*n - not viable i.e., the bacterial culture was killed/inactivated

Table 4: *In vitro* antimicrobial activity testing of hand rub disinfectant formulation F3 and F4 on various microorganisms

Test organisms	Viability/Inhibition of organisms in undiluted form of hand rub disinfectant after a contact time in seconds (same for F3 and F4)					
	45	60	120	180	240	360
E. coli	n	n	n	n	n	n
Salmonella typhi	n	n	n	n	n	n
Staphylococcus aureus	n	n	n	n	n	n
Shigella flexner	n	n	n	n	n	n
Pseudomonas aeruginosa	n	n	n	n	n	n
Candida albicans	n	n	n	n	n	n
Aspergillus niger	n	n	n	n	n	n

*n - not viable i.e., the bacterial culture was killed/inactivated

of tween 80. Thus it can be concluded that, amongst all these four formulations, F4 was the best formulation.

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Source of Support: Nil, **Conflict of Interest:** None declared.