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THE EVOLUTION OF BENZALKONIUM CHLORIDE AND ITS IMPACT ON MICROBIAL RESISTANCE

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ABSTRACT

This study provides a thorough validation of a UV spectroscopy approach for the quantification of benzalkonium chloride (BKC). Through examinations of selectivity and specificity, the study validates the method's reliability and validates its capacity to differentiate BKC from typical sample interferences. A strong calibration curve is established by linearity investigations over the anticipated concentration range. Recovery studies and other accuracy evaluations show how accurate the approach is at determining the real BKC concentrations. The robustness of the method is tested by systematic fluctuations in essential parameters, and its sensitivity to low BKC concentrations is demonstrated by the limits of detection and quantification. The UV system's correct operation is guaranteed by system suitability requirements. Evaluations of stability confirm that BKC is robust in solution under various storage circumstances. Strict documentation follows global guidelines, guaranteeing that the technique is appropriate for precise BKC analysis in industrial and pharmaceutical applications.

Keywords: Benzalkonium Chloride, Quaternary Ammonium Compounds (Qacs), Antibacterial, Antimicrobial, UV-Spectroscopy.

I. INTRODUCTION

Early in the 20th century, benzalkonium chloride (BAC) was first produced and identified. Its synthesis and discovery history can be linked to the advancement of quaternary ammonium compounds (QACs). Tertiary amines were quaternized with alkyl halides to create quaternary ammonium compounds for the first time in the late 19th and early 20th centuries. In particular, benzyl chloride was quaternized with dimethylamine or other tertiary amines to create benzalkonium chloride. Early chemical literature reported the synthesis of BAC and described a number of preparation and purification techniques. It was through empirical observations in the laboratory that the antimicrobial properties of quaternary ammonium compounds, such as benzalkonium chloride, were discovered. Early scientists discovered that QACs had strong biocidal properties against bacteria, fungi, and viruses. This led to their investigation as antiseptics and disinfectants in medical and cleaning settings. As benzalkonium chloride's antimicrobial qualities were discovered, large-scale manufacturing techniques were created to satisfy the expanding need for antiseptics and disinfectants .Benzalkonium chloride's broad-spectrum antimicrobial activity and compatibility with a variety of formulations led to its widespread use in a number of industries, including pharmaceuticals, personal care products, and household cleaners. The synthesis and discovery of benzalkonium chloride are referenced in various historical accounts, but early chemical literature and patents from the early to mid-20th century frequently shed light on the creation and application of quaternary ammonium compounds, such as BAC. Examples of early 20th-century scientific publications, historical writings, and patents that are pertinent to the topic may provide insight into the synthesis, discovery, and initial uses of benzalkonium chloride. Quaternary ammonium compounds (QACs) include benzalkonium chlorides (BACs), also referred to as alkyl dimethyl benzyl ammonium chlorides, alkyl dimethyl (phenylmethyl) quaternary ammonium chlorides, ammonium alkyl dimethyl (phenylmethyl) chlorides, or ammonium alkyl dimethyl benzyl chlorides. Typically, they are sold as a blend of substances having varying alkyl chain lengths, from C8 to C18, with C12 and C14 derivatives having higher biocide activity.

When BACs were first reported by Gerhard Domagk in 1935, they gained popularity as zephiran chlorides and were promoted as excellent and promising antiseptics and disinfectants. The United States' Environmental Protection Agency (EPA) received the first product containing BACs in 1947. They have since been incorporated into many different over-the-counter and prescription products. Applications include industrial,

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clinical, agricultural, and domestic. In the home, these products find use in fabric softeners, ophthalmic solutions, nasal delivery medications, and personal hygiene and cosmetic products like shampoos, conditioners, and body lotions. In addition, BACs are frequently found as active ingredients in disinfectants that are used in commercial, industrial, institutional, and agricultural contexts.

Additional registered uses for BACs in the United States include applications on indoor and outdoor surfaces (walls, floors, toilets, etc.), agricultural tools and vehicles, humidifiers, water storage tanks, products for use in residential and commercial pools, decorative ponds and fountains, water lines and systems, pulp and paper products, and wood preservation.

The use of BACs for a variety of purposes, many of which inevitably produce and release residual biocide, can lead to the development of environments where microbes are under selective pressure to become resistant to these substances. Tracking outbreaks that are typically linked to the misuse or incorrect dilution and storage of disinfectants and antiseptic solutions has shown how bacteria can survive and grow in BACs. In fact, throughout the course of four decades, BACs were linked to multiple outbreaks, which prompted a number of recommendations to stop using them as an antiseptic.

The use of BACs as antiseptics has long been associated with concerns; as early as the 1960s, researchers found resistant strains that could survive in BAC solutions (0.1 to 0.4%). Bacteria are known to be able to adapt and become more resistant to harmful substances; this is a phenomenon that has been repeatedly demonstrated for BACs. When selection pressure is removed from evolved strains, adaptive mutations that promote tolerance or resistance are often stable at the population level and continue to be seen. It has been shown that bacteria can evolve to survive to BAC concentrations similar to those found in the environment and in consumer products, despite the fact that the reported concentrations vary depending on the study and the bacterial genera.

II. BKC DRUG PROFILE

Benzalkonium chloride (BKC):

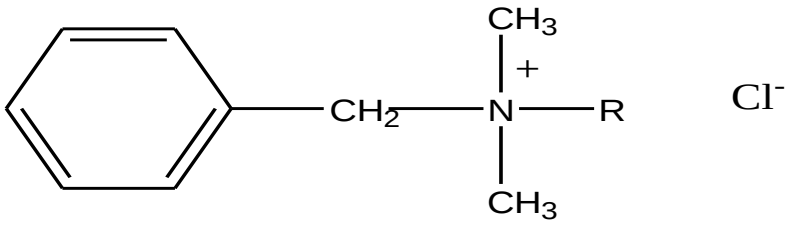

Figure 1: Benzalkonium chloride

IUPAC name: - Mixture of alkylbenzyldimethylammonium chlorides

Molecular formula: - C17H30CIN

Molecular weight: - 311.938 g/mol

Solubility: - Ethanol and water soluble. Furthermore, insoluble in diethyl ether.

Melting point: - 149-153^{0}C

Boiling point: - 250-260^{0}C

λmax: - 277.5

The compound was found to be soluble in water and hence water was used as solvent for performing analysis of this analog.

Pharmacological aspects of BKC

Benzalkonium chloride solutions are generally categorized as biocidal agents with relative long durations of action. Their spectrum of activity has been demonstrated against bacteria, to some viruses, fungi, and protozoa, although bacterial spores are treated as being resistant to the agent. Additionally, the agent generally shows more activity against gram-positive than gram-negative bacteria. Finally, solutions of benzalkonium chloride are bacteriostatic or bactericidal based on their concentration. Bacteriostatic agents act to prevent further growth of bacterial organisms that are present while bactericidal agents function to kill bacteria that are present. In general, the activity of the agent is not largely affected by pH, but such activity does increase substantially at higher temperatures and prolonged exposure times. Like other quaternary ammonium compounds,

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benzalkonium chloride is probably poorly absorbed and mostly eliminated in feces due to its large, positively charged molecular structure.

Antibacterial mechanism of BKC

The mode of action of QACs, including BACs, entails the alkyl chains' perturbation and disruption of the membrane bilayers as well as the charged nitrogen disruption of the membrane's charge distribution. Accordingly, a variety of processes, many of which are connected to the cell membrane, may lead to susceptibility to BACs. Changes in the overall composition of the membrane, downregulation of porins, overexpression or modification of efflux pumps, transposon element and stress factor gene transfer horizontally, biofilm formation, and biodegradation are some of the mechanisms suggested in the literature.

Decreased susceptibility to BACs has long been linked to modifications in the composition of the membrane. It has been demonstrated that the phospholipid and fatty acid compositions of resistant and susceptible strains of P. aeruginosa differ. Additional research has shown that Bacillus cereus exposed to BACs induced genes related to fatty acid metabolism and altered the membrane's fatty acid composition. But the authors didn't assess if exposed strains showed a tolerant phenotype. It was demonstrated that an E. Coli strain that was less susceptible to BACs had a different lipopolysaccharide composition than the susceptible strain. It was recently proposed that Pseudomonas strains could stabilize the membrane charge by upregulating the expression of the gene responsible for polyamine synthesis, thereby partially adapting to BACs.

It has been proposed that fewer BACs entering the system contribute to a lower susceptibility to the biocide. Since porins are thought to be involved in the adsorption of QACs, downregulating porins could theoretically result in reduced susceptibility. Accordingly, Pseudomonas and E. coli strains that are less vulnerable to BACs have been linked to the downregulation of genes encoding multiple porins. The susceptibility of the strain to BACs was reduced when the porin OmpF level in the E. coli membrane was reduced.

For Mycobacterium smegmatis, a causal relationship was established between a disinfectant product containing BACs and the downregulation of porins; Msp porin knockout mutants were less sensitive to the biocide than the wild type. However, the degree to which the observed effect can be attributed to BACs, other ingredients in the formula, or the mixture is limited by the authors' use of a disinfectant formulation. To support the connection between the downregulation of porins and tolerance to BACs, more research is needed.

BKC as an Antimicrobial agent

By rupturing the hydrophobic barrier of the corneal epithelium and breaking cell-cell junctions, BAK has been proposed to improve drug penetration into the cornea and consequently the anterior chamber. This would lead to a greater concentration of the drug in the tissue and aqueous solution, which would improve efficacy.

When BAK is used as a preservative in antibiotic drops, its disruption of the corneal barrier may enhance the antimicrobial effect of the drop's "active ingredient." In addition, BAK's antimicrobial efficacy may work in concert with the antimicrobial properties of the drop's "active ingredient." Notably, several trials comparing preserved and preservative-free formulations of various glaucoma medications found no difference in intraocular pressure (IOP) reduction. These studies highlight the clinical equivalency between some BAK-preserved and unpreserved ophthalmic medications. As a result, switching from a BAK-preserved to a BAK-free regimen is frequently possible in specific clinical situations without impairing IOP control.

Procedure of synthesis of BKC

Prepare a solution of dimethylamine by dissolving it in water. Ensure the concentration is appropriate for the reaction. Add benzyl chloride to a suitable reaction vessel that has a stirring device. Stirring constantly, gradually pour the dimethylamine solution into the reaction vessel. Since the reaction is exothermic, you might wish to use a water bath to cool the reaction vessel in order to regulate the temperature, if needed. Dimethylamine and benzyl chloride combine to form benzalkonium chloride and other reaction byproducts as the reaction progresses. To make sure the reaction is finished, keep stirring the reaction mixture after the addition is finished. Once the reaction is finished, add a suitable base, like sodium hydroxide, to neutralize any excess acid that may have formed. Continue doing this until the pH reaches the desired range. Using a separatory funnel, remove the benzalkonium chloride from the reaction mixture. The organic phase will contain the benzalkonium chloride. If purification is necessary, the crude benzalkonium chloride can be purified by

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methods such as distillation or recrystallization. If required, use a drying agent or lower pressure to dry the purified benzalkonium chloride. Keep the benzalkonium chloride out of direct sunlight and moisture in an appropriate container.

Benzalkonium Chloride Concentrated Solution 50

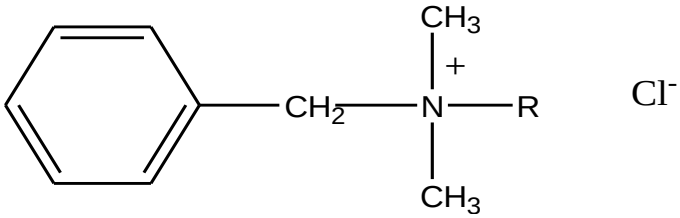
Concentrated solution 50 of benzalkonium chloride is a solution of water with an amount of $C_{12}H_{25}$ and C14H29. This appears as $[C_6H_5CH_2N(CH_3)2R]$ Cl. It includes benzalkonium chloride ($C_{22}H_{40}$ ClN:354.01) at an amount of not less rather than 50.0 w/v% and not over 55.0 w/v%.

Description: -

Benzalkonium Chloride Concentrated Solution 50 is a colorless to pale yellow liquid with a pronounced odor. It dissolves very well in water and ethanol, but nearly unsolvable in diethyl ether (95). A solution that contains fluid added to it bubbles violently if shaken.

Benzalkonium chloride solution: -

Benzalkonium chloride is an example of cationic surfactant, frequently referred to as alkyl dimethyl benzyl ammonium chloride, BZK, BKC, BAC, and ADBAC. It belongs to the category of quaternary ammonium compounds as an organic salt. Its applications are mostly in three areas: phase transfer agent, cationic surfactant, and biocide. Alkyl benzyl dimethylammonium chlorides (ADBACs) are made up of alkyl groups that have various even-numbered alkyl chain lengths. Since benzalkonium chloride is hygroscopic, it easily absorbs moisture or, when heated, turns into a transparent molten substance. This characteristic improves its effectiveness as a strong antimicrobial and fungicidal agent, preventing the slow growth of bacteria in multidose containers. BKC was originally readily accessible as a germicide in the 1910s, so by the 1940s, it was used extensively. In the 1940s, the ophthalmic industry used BKC for the first time as a hard contact lens solution. Since then, it has been used for over-the-counter synthetic tear solutions as antiglaucoma medicines. In optical preparations offered in the European Union, subsequently appears the main preservative was BKC. BKC is used as a preservative in around 74% of optic preparations. In many pharmaceutical products intended to nasal delivery for numerous presentations in respiratory use permitted on EU markets, BKC is also utilized as an antibacterial preservative. In pharmaceutical preparations, BKC is often employed at a concentration of 0.002% to 0.02%, while in some cases, depending on several parameters in ophthalmic formulations, it may reach 0.2%. Medical items containing BKC may be used orally, orally mucosal, rectal, cutaneously, intramuscularly, intra-articularly, subcutaneously, vaginally, or auricularly. In the chemical industry, BKC is primarily used as a biocide, phase move agent, and a cationic surfactant was designed to be a reliable method of birth control. Treatment for Lozenges-related minor infections of the oral cavity and throat involves the use of benzalkonium chloride. Because it stops the growth of microorganisms and kills them, BKC is commonly used in antiseptic moisturizers, antimicrobial hand wipes, anti-itch creams, and ophthalmic preparations. According to FDA guidelines in the US, first aid products should include BKC at safe and effective doses of between 0.1 and 0.2%. Different analytical techniques have been developed to estimate BKC in preparations for eye exams.



Benzalkonium chloride

Validation

The UV spectrophotometric analytical method has been verified using a number of parameters, such as linearity, robustness, precision, accuracy, as well as limits of identification and quantification.

III. CONCLUSION

The development of benzalkonium chloride (BKC) from its early synthesis to its extensive use in many different industries emphasizes the importance of this compound as an antimicrobial agent. Its proven effectiveness and

historical significance notwithstanding, questions have been raised about its continued use as an antiseptic due to outbreaks linked to misuse and concerns about microbial resistance.

The pharmacological profile of BKC is marked by its poor absorption and broad-spectrum antimicrobial activity, which highlights its adaptability to a variety of formulations. Bacterial adaptations have reduced susceptibility, though, so continued study and regulatory oversight are required to guarantee its effectiveness and safety.

Hygroscopic in nature, BKC is a versatile biocide that is well-liked in pharmaceuticals, ophthalmic solutions, and other consumer products because it increases its antimicrobial efficacy. The use of analytical methods like UV spectrophotometry is essential to guaranteeing the proper and secure dosage of BKC in these mixtures.

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