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Non Standard Evaluation of the Efficacy of Gel- and Liquid-Alcohol-Based Hand Rubs against *Staphylococcus aureus* by Chemical and Biological Electronic Scanning Microscopic Techniques

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Abstract: Alcohol sanitisers are the most commonly used disinfectants and antiseptics in health care settings. Their efficacy is affected by concentration and contact time. The contact time can be studied with chemical kinetics that relates concentration, time and temperature in the Arrhenius equation based on kinetic molecular theory. The main objective of this paper was to present experimentally obtained results of the evaporation kinetics of liquid and gel alcohol based-hand rub sanitisers at 30 °C. The evaporation reaction of alcohol-based hand rubs follows a first order reaction and the rate constant is equal to 0.00066 and 0.001615 for gel and liquid sanitisers, respectively. The half-life of the gel and liquid rubs were 17.5 and 7.12 hr, respectively. The low value of the rate constant and high half life time of the gel-form sanitiser are related to it porous structure. The high half life time value caused by the effect of the porous structure expected to increase the contact time to inhibit the S*taphylococcus aureus* activity. In contrast, the electronic scanning microscope study of the gel and liquid biofilms of the alcohol based-hand rubs showed that cells grown in the liquid alcohol-based hand rub (AHR) medium exhibited more indentation and produced more cell debris than cells grown in the gel AHR. These configurations suggest that liquid AHRs are more effective at destroying *Staphylococcus aureus* cells than gel AHRs. Therefore, liquid AHRs are more efficient sanitisers than gel AHRs.

Key words: Health Care Settings · Sanitisers · *Staphylococcus aureus* · Evaporation · Rate Constant · Electronic Scanning Microscope

INTRODUCTION

Alcoholic compounds have many applications in health care settings (HCS): they are used as solvents, stabilisers and sanitisers. The traditional liquid form was originally used in HCS. Subsequently, gel-based and non-aerosol alcohol-based foam were introduced into HCS in the 1980s and 2006, respectively [1]. The main objective of using alcohol-based hand rub (AHR) sanitisers is to achieve hygienic hand disinfection, or treat hands post-contamination and disinfect surgical hand, or treat hands preoperative [2]. Strong evidence showed that the use of AHRs to reduce the transient and resident flora on the hands [3,4] reduced the incidence of healthcare-associated infections [5-8] with special regards to Methicillin Resistant *Staphyloccus aureus* (MRSA) [3]. Alcohol disinfectants acts via: the disruption of membrane function or structure of microbes, interference with cell division and/or steady-state growth, inhibition of nutrient transport via membrane-bound ATPases, alteration of fatty acid composition and protein synthesis and reduction in microbial intracellular pH [9]. The efficiency of sanitisers (whether in liquid or gel form) depends on the intrinsic biocidal activity and the concentration of the sanitiser, the contact time, the hardness of the water used to dilute the sanitiser and the type and number of microorganisms present [10,11].

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Table 1: Active and inactive ingredients of liquid and gel form sanitisers

Form	Liquid form	Gel form
Active ingredients	Ethanol 70% v/v	Ethanol 70% v/v
Non active ingredients	Purified water	Purified water
		Thickening agent: Acrylates/ C10-30 Alkyl Acrylate Cross-polymer
		Moisturizing agent: Glycerin
		Excipients

The rates of most chemical reactions are classified experimentally as zero, first and second order reactions according to the species involved in the reaction [12]. The evaporation rate of an AHR usually follows zero-order kinetics. However, an ethanol/water mixture (50/50) follows first order kinetics [13-16].

The second factor that affects the efficiency of an ethanol sanitiser is its concentration. Concentrations of alcohol less than 70%were significantly less effective than higher concentrations (such as 75%). An ethanol concentration higher than 60% is generally safe and effective for topical use on hands [17,18].

The active ingredients of AHRs are ethanol, 1-propanol, 2-propanol or a combination of two of these alcohols [19,20]. The combination of different alcohols proved that 70% ethanol alone provided better sanitising results than the combination of 70% ethanol and propanol. In addition, different inactive ingredients, such as moisturisers and other additives, could affect the effectiveness of the formulations (Table 1) [21-23].

Because of the higher efficacy of AHRs [3,24] and the disadvantages of alternatives such as antimicrobial soaps (decreased dermal tolerance [3,25], higher potential for impaired efficacy due to an incorrect performance of the procedure, need for a wash basin and longer time spent on the cleaning procedure) [26], AHRs are favoured by the Centers for Disease Control (CDC) and the World Health Organisation (WHO) guideline on hand hygiene in HCS [4,27].

The hypothesis of this work is simply stated as " liquid is better than gel AHRs". To test our hypothesis, this work will determine the evaporation rate of both forms of AHRs kinetically. In addition, electronic scanning microscopic micrograph will compare the effect of liquid and gel AHRs on the identity of *Staphylococcus aureus* pathogenic cell.

MATERIALS AND METHODS

Kinetic Study: A commercial formulation of a sanitiser containing 70% (v/v) ethanol was used in this experiment. The formulation weights were maintained at different time intervals at a constant temperature (30°C). Ten samples of a specific volume (50 ml) of the formulation were placed in beakers, weighed and placed in a hot bath at 30°C. The samples were weighed after 15, 30, 45, 60, 75, 90, 105, 120, 135 and 150 min. The weight after evaporation and the change in weight over time was recorded and the data were plotted as zero and first order reactions.

Scanning electronic microscope. *Staphylococcus aureus* (strain no. 29213 obtained from Kuwait Institute for Medical Specialization, Faculty of Laboratory Medicine–Kuwait) were harvested after 24 hours. The bacterial cells were suspended separately in liquid and gel AHRs in 0.1 M phosphate buffer (pH 7.2) at room temperature. The bacterial cells were washed with phosphate buffer and prepared for the scanning microscope. A dense suspension of washed cells was transferred to a grid. The cells were dehydrated with an ethanol gradient and subjected to critical point drying. Subsequently, the samples were mounted on aluminium sample holders, sputter-coated with platinum and inspected with a scanning microscope (JOEL ESM model no.JSM-6300) at 20 to 30 kV.

RESULTS AND DISCUSSION

The mass loss of AHR sanitisers was determined by weighing 50.0 ml of sanitiser in a beaker before and after evaporation for specific times. The data collected for mass and time were plotted for two suggested kinetics. The first suggestion was plotted as a zero order reaction (*mass0 - mass$_t$* vs. *time*). The second suggestion was plotted as a first order reaction ($1/mass_t$-$1/mass_o$ vs. *time*) [28,29]. The zero and first order expressions are plotted in figures 1 and 2, respectively. According to figures 1 and 2, the best fit line values (R^2) for the gel and liquid are determined in zero order plots. However, the first order reaction is supported by the literature [13]. In particular, the efficiency of ethanol depends on the concentration of the sanitiser and contact time. The contact time can be studied by chemical kinetics, the area of chemistry concerned with the rates of reactions. The rate of a reaction is the change in the concentration of the reactant with time; the rate depends on the nature of the reactants, the physical state of the reactants, the concentration of the reactants, the temperature at which the reaction occurs and the presence of a catalyst. According to kinetic molecular theory, the rate of a reaction increases with increasing number of effective collisions between molecules and the specific minimum kinetic energy that activates the chemical reaction (*Ea*).

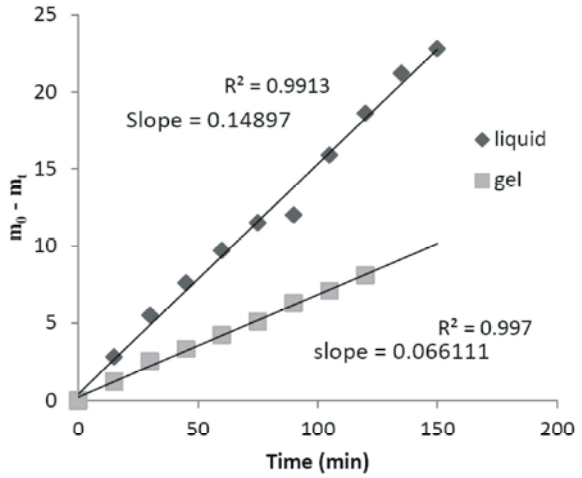

Fig. 1: Zero-order plot of the mass loss for the gel and liquid AHRs as a function of time. The best fit lines (R^2) for the gel and liquid are shown.

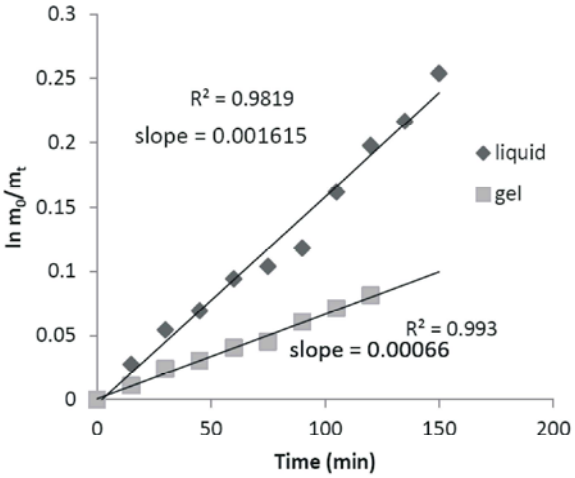

Fig. 2: First-order plot of the mass loss for the gel and liquid AHRs as a function of time. The best fit lines (R^2) for the gel and liquid are shown.

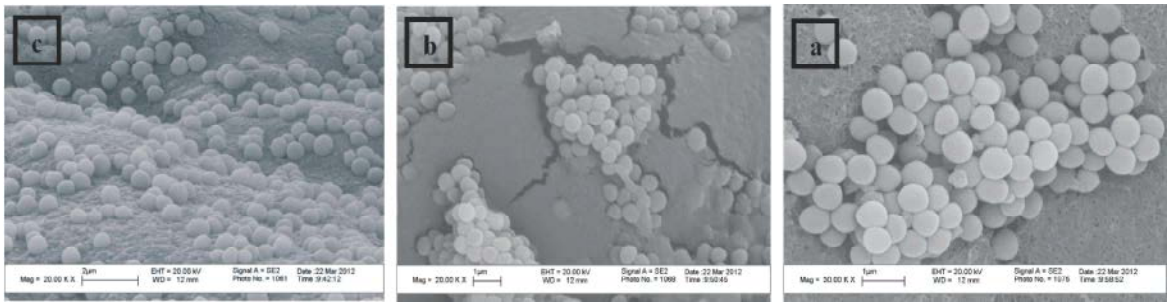

Fig. 3: Effect of the gel and liquid AHRs on the micromorphology of *Staphylococcus aureus*. Representative scanning micrographs of *Staphylococcus aureus* grown in the absence of AHRs(a), in the presence of liquid AHRs (b) and in the presence of gel AHRs (c) are shown.

These factors can be found in the Arrhenius equation: $k = Ae^{-Ea/RT}$, where k is the rate constant, E_a is the activation energy, R is the gas constant, T is the absolute temperature and A is the frequency factor. The activation energy is inversely proportional to the rate of the reaction as the fraction of molecules that possess the required energy is smaller [12]. A comparative kinetic study of the alcohol evaporation revealed that the times required for half of the concentration to evaporate ($t_{1/2}$) were 17.5 hr for the gel form and 7.12 hr for the liquid form. The experimentally determined evaporation rate constants for the gel and liquid AHRs were $k = 0.00066$ min^{-1} and $k = 0.001615$ min^{-1}, respectively. The lower evaporation rate constant for the gel (approximately 10 times lower) was attributed to the porous structureof the gel, which delays the evaporation rate. The liquid form of the AHRs requires less kinetic energy to activate evaporation (E_a), whereas the gel form of the AHRs requires more energy (E_a) to activate evaporation. The variation in (E_a) was attributed to the presence of pores and to the nature of the evaporative mechanism; indeed, the AHRs in the gel form need a longer contact time to affect the *Staphylococcus aureus* pathogen than the liquid form.

Porosity is a significant factor that reduces the exposed surface of the AHR and, consequently, reduces the rate of evaporation. Large pore volumes are generated for gel AHRs due to several effects: the lower surface tension of ethanol reduces the capillary forces during drying, thus leading to less collapse of the gel network; changes in the wet gel pore size distribution; and changes in the contact angle as the nature of the pore surface changes. The drying of gel AHRs in a wet state leads to significant structural rearrangements, causing a larger surface area, smaller pore size and narrower pore size distribution due to both esterification of the pore surface and depolymerisation of the gel matrix. These structural rearrangements are reversed when the AHR gel has higher water content. Controversially, the dry state of AHR gels shows an appositely different structural characterisation. When evaporation begins to expose the gel phase, the

ethanol/water mixture tends to spread over the gel phase. As the ethanol/water mixture stretches to cover the gel, tensile stress appears in the ethanol/water mixture and compressive stress is imposed on the gel network. The gel network is very flexible and can collapse into a liquid mixture that is aspirated under the surface of the mixture; as a result, most of the area covered by the sanitiser is not exposed to the AHRs and will therefore not affect *Staphylococcus aureus.* The surface area of the mixture and the volume of the pores play an important role in initiating the gel collapse and therefore affect drying. In the wet alcoholic state, the gel network is characterised by a decrease in the surface area of the mixture, large pore sizes and a broader distribution of pore sizes. In contrast, in the dry alcoholic state, the gel network is characterised by an increase in the surface area of the mixture, a reduction in pore sizes and a narrowing of the distribution of pore sizes [17,30,31]. These characteristics strongly impact contact time and, therefore, the inhibition of *Staphylococcus aureus*.

The ESM micrographs of *Staphylococcus aureus* grown in gel- and liquid-form AHRs in figure 3 show the morphological differences in *Staphylococcus aureus* cells grown under different conditions. Cells grown in the absence of AHRs had a more intact, normal, smooth and spherical appearance. In contrast, cells grown in the liquid AHR medium exhibited more indentation and produced more cell debris than those grown in the gel AHR medium. These configurations suggest that liquid AHRs are more effective at destroying *Staphylococcus aureus* cells, which is incongruent with the results related to evaporation rate.

CONCLUSION

The kinetic study of AHRs containing a 70% (v/v) ethanol/water mixture showed that the evaporation follows a first order reaction. The evaporation rates of the gel and liquid AHRs were 0.00066 and 0.001615 min^{-1}, respectively. The half-lives required for half of the concentration of the gel and liquid AHRs to evaporate at 30°C were 17.5 and 7.12 hr, respectively. This study revealed that gel AHRs had lower evaporation rates and higher half-lives than liquid AHRs. The pores of the gel form minimise the number of molecules exposed at the surface and decrease the evaporation rate. Increasing the size of the pores of the AHRs leads to a significant decrease in the evaporation rate; however, the surface area affected by the sanitiser also decreases. Increasing the size of the pores will increase the area of the surface that is not affected by the sanitiser. Consequently, the efficiency of gel AHRs at affecting *Staphylococcus aureus* is lower than that of liquid AHRs. The ESM study reinforced our hypothesis that liquid AHRs are more efficient sanitisers than gel AHRs.

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