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ORIGINAL ARTICLE

Physicochemical characterization of reusable facemasks and theoretical adhesion by a challenged bacterium

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ABSTRACT

Background and Objectives: Adhesion of microorganisms on facemask surfaces is a major problem that produces contamination of the mask wearer either by inhalation or by direct contact. Generally, physicochemical properties of the material and the microorganism are responsible for this adhesion and are also reported to influence the filtration efficiency of facemasks. However, theses surface proprieties and their effect on particles attachment on facemask materials remain poorly documented. The purpose of this study was to investigate the physicochemical properties of seven facemasks and evaluate the influence of these characteristics on the adhesion of *Sataphylococcus aureus.*

Materials and Methods: Physicochemical properties is done by contact angle method and scanning electron microscopy while theoretical adhesion of *S. aureus* is done according to XDLVO approach.

Results: The obtained results showed that all masks have a hydrophobic character. The electron donor and electron acceptor parameters change depending on each mask. Chemical analysis demonstrates the presence of two chemical elements (carbon and oxygen). Predictive adhesion demonstrate that *S. aureus* has an attractive behavior towards the masks used but the potential of adhesion is not the same.

Conclusion: Such information is valuable to understand attachment of biological particles and to contribute in the inhibition of this attachment.

Keywords: COVID-19; Masks; *Staphylococcus aureus*; Surface properties

INTRODUCTION

The COVID-19 pandemic caused by the Sars-Cov-2 virus first appeared in Wuhan, China in December 2019 is posing a huge global health threat (1). Public health and social measures are being implemented globally to limit the spread of COVID-19 and reduce its mortality and morbidity (2). Therefore, the transmission of Covid-19 virus can occur by direct contact with an infected person or indirectly through

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an object already infected with the virus (1). This viral transmission led to the use of facemasks as an appropriate tool to combat environmental risks (3) and to avoid viral respiratory diseases like Sars-Cov-2 (1). Reusable knitted fabric masks are among the most used masks during the mask shortage. These masks are considered as barrier masks. They are formed by a combination of fibers that allow the retention and collection of microorganisms. Therefore, this collection can cause the development and the survival of harmful particles on the filter materials and constitute a risk through skin contact with the contaminated filter or through inhalation of airborne particles (4). The adhesion of microorganisms or particles on the surface of the facemask is the step that precede filtration of particles through the mask. The small particles suspended in the air have a disordered and random trajectory (Brownian agitation) and under the effect of this Brownian agitation, they can be exposed to a fiber of the filter and adhere to it. The adhesion of a microorganism on a surface is a process that calls for several energetic and physicochemical interactions between the support and the microorganism (5-9). Moreover, physicochemical proprieties of facemask materials are reported to influence filtration efficiency (10).

In the interface between fiber and the particles, exists, an intermolecular force of attraction called Van der Waals force. Among these interactions we find dispersive or Van Der Wals interactions, electrostatic interactions, non-dispersive interactions including acid-base component, hydrogen bonds and hydrophobicity of the surface (11, 12). In addition, hydrophobicity, electron donor/acceptor parameter and surface energy are known to be significant factors in the adhesion process. The predictive adhesion on the surface of the masks is performed using the XDLVO approach, in fact it is an approach derived from the DLVO (derjaguin, landau, verwey, and overbeek) approach. The XDLVO approach considers Van Der Wals interactions, electrostatic interactions and acid-base interactions in the quantification of the total energy of interaction between two entities. However, the XDLVO theory does not always provide a clear prediction of bacterial adhesion (13, 14) because of the biological, chemical and structural complexity of bacterial cells (9). Nonetheless, it gives appropriate answers to explain the experimental findings of adhesion (15, 16).

S. aureus is a bacterium known by its pathogenicity, its strong adhesive power to different surfaces (8) and its employment as challenging bacterium to evaluate the filtration efficiency of facemasks in international standards test, were among the reasons why it was considered for the current study. To our knowledge, information about the physicochemical properties of facemasks surfaces and its relation with attachment of particles is poorly documented. The purpose of this work was, to investigate the physicochemical properties of seven facemasks by sessile drop method, analyze the chemical composition of these surfaces and the visualization of the morphological structure by SEM and Energy Dispersive X-Ray Analyzer (EDX). The predictive adhesion of *S. aureus* ATCC25923 on the surface of the facemasks was conducted according to XDLVO approach.

MATERIALS AND METHODS

Bacterial strain, growth conditions and contact angle mesurements. For the preparation of the bacterial culture, *S. aureus* ATCC25923 is cultivated in the liquid medium of Luria Bertani during 24 h at 37°C, after the bacteria is washed twice by a solution of KNO_3 of 0,1M and centrifugation during 15 min at 8400×g then suspended in the KNO_3, 0.1M solution. The already washed bacteria placed in the KNO3 solution is poured into a 0.45 um cellulose acetate filter and filtered under negative pressure. Afterward, the filters have been carefully deposited on a glass support. Contact angles were measured in triplicate separately (17).

Preparation of mask surfaces and contact angle measurements. In this work, seven reusable masks commercialized in Morocco were used. The purchased masks nominated as follows (A, B, C, D, E, F, and G) are cut into pieces of fabric by the following dimension (6 cm^2) and a droplets of 5 µL of liquid probe was deposited perpendicularly on them and the contact angles were measured in triplicate separately.

Contact angles measurements. The measurement of the contact angle is carried out by a goniometer (GBX instruments, France). To measure the contact angle, it is necessary to use certain liquids such as diiodomethane (99%), formamide (99%), and water. These liquids have known constants (Table 1). The measurement of the surface tension for the acid base (AB) and Lifshitz Van-der Waals (LW) components is obtained by measuring the contact angle of a solid surface using the three liquid probes and substituting the value obtained in the equation of Young Dupré equation (18):

$$y_L\left(COS\theta+1\right)=2\left(\sqrt{y_s^{Lw}y_s^{Lw}}\sqrt{y_s^{+}y_s^{-}}+\sqrt{y_s^{-}y_s^{+}}\right) \quad (1)$$

Where:

θ: the contact angle

γLW : the Van der Walls free energy

γ+: the electron acceptor parameter

γ–: the electron donor parameter

(S) Represent solid surface and (L) represent liquid phase.

The free energy of the surface (ΔG_{iwi}) represents the degree of hydrophobicity of a given material (i) and it represents the free energy of the surface between two entities of the material (i) when plunged into water. When the interaction between the two entities is powerful than the interaction of each entity with water, the ΔG_{iwi} has a negative value and the material is qualified as hydrophobic. In contrast, if the interaction is weak, the ΔG_{iwi} is positive and the surface is considered hydrophilic.

ΔG_{iwi} is calculated using the following formula:

$$\Delta G_{iwi}=-2y_{iw}=-2\left[\left(\sqrt{y_i^{Lw}-y_w^{Lw}}\right)^2+2\left(\left(\sqrt{y_i^{+}y_i^{-}}\right)+\left(\sqrt{y_w^{+}y_w^{-}}\right)-\left(\sqrt{y_w^{+}y_i^{-}}\right)-\left(\sqrt{y_i^{+}y_w^{-}}\right)\right)\right] \quad (2)$$

Theoretical prediction of adhesion on facemasks using XDLVO theory. Predictive adhesion between colloids and surfaces can be estimated according to the XDLVO approach by measuring the total energy of interaction (ΔG^{Total}). The sum of liftshiz Van-der-Waals (LW), acid base (AB) and electrostatic double layer (EL) interaction represents the total free energy of interaction between substrate and bacterial cell across water (19):

$$\Delta G^{Total}=\Delta G^{Lw}+\Delta G^{AB}+\Delta G^{EL} \quad (3)$$

Where:

$$\Delta G^{Lw}=\left(\left(y_M^{Lw}\right)^{1/2}-\left(y_S^{Lw}\right)^{1/2}\right)^2-\left(\left(y_M^{Lw}\right)^{1/2}-\left(y_L^{Lw}\right)^{1/2}\right)^2-\left(\left(y_S^{Lw}\right)^{1/2}-\left(y_L^{Lw}\right)^{1/2}\right)^2 \quad (4)$$

And:

$$\Delta G^{AB}=2\left[\left(y_L^{+}\right)^{1/2}\left[\left(y_M^{-}\right)^{1/2}+\left(y_S^{-}\right)^{1/2}-\left(y_L^{-}\right)^{1/2}\right]+\left(y_L^{-}\right)^{1/2}\left[\left(y_M^{+}\right)^{1/2}+\left(y_S^{+}\right)^{1/2}-\left(y_L^{+}\right)^{1/2}\right]-\left(y_L^{-}y_S^{+}\right)^{1/2}-\left(y_L^{+}y_S^{-}\right)^{1/2}\right] \quad (5)$$

ΔG^{EL} is the electrostatic double layer interaction, ΔG^{EL} is neglected when bacteria are treated with high ionic strength (0.1 M) of KNO_3 solution.

According to this theory the value of ΔG^{Total} is an indicator that predicts whether adhesion will be favorable ($\Delta G^{Total}<0$) or unfavorable ($\Delta G^{Total}>0$).

Scanning electron microscopy and energy dispersive X-ray analyzer. The morphological structure of the masks is visualized by SEM. It scans a focused electron beam over a selected area of the surface of the sample to generate an image. The chemical elements of the surface of the masks and their quantity are done by EDX analyzer.

RESULTS

Characterization of materials and bacterial surface. The surface properties of the masks obtained by contact angle method are presented in Table 2. The results show that the seven masks (A, B, C, D, E, F and G) have a higher θwater of (125°;137.8°;131°;125.4° ;71.77°;104.1°;101.67°) respectively, with a negative surface free energy ($\Delta G_{iwi}<0$) which shows very clear hydrophobic character.

Moreover, the electrons donor and electrons acceptor parameters are also studied and have shown that the electron donor character varies between (0.2mJ/m^2 to 11.43mJ/m^2) while, the electron acceptor parameter varies between (0.07mJ/m^2 to 10.67mJ/m^2).

As shown in Table 3, *S. aureus* ATCC25923 has a hydrophilic surface (θwater =19.9°), high electron donor property (53 mJ/m^2) and a very limited electron acceptor parameter (1.7 mJ/ m^2).

Predictive adhesion of *S. aureus* ATCC25923 on the surface of the facemasks. Our study evaluated also the potential of *S. aureus* ATCC25923 to adhere on the fabric of the masks. The understanding of inter-

Table 1. Surface energy of contact angle liquids

Liquid	γ^{LW}	γ^{+}	γ^{-}
Water:	21.8 mJ/m^2	25.5 mJ/m^2	25.5 mJ/m^2
Formamide	39.0 mJ/mm^2	2.3 mJ/m^2	39.6 mJ/m^2
Diiodomethane:	50.5 mJ/mm^2	0.0 mJ/m^2	0.0 mJ/m^2

Table 2. Contact angles of water (θw), formamide (θF), diiodomethane (θD), Lifshitz-Van der Waals (γLW), electrons donor(γ–), electrons acceptor (γ+) parameters obtained with the seven facemasks.

Masks species	Contact angles (°)			Surface tension: components and parameters ($mJ\ m^{-2}$)			ΔG_{iwi} ($mJ\ m^{-2}$)
	θ diiomethane	θ formamide	θ water	γ (LW)	γ (+)	γ (-)	
A	103.9 (1.4)	132.9 (0.2)	125 (2.4)	7.3	3.2	5.6	-42.3
B	107.4 (1.4)	127.3 (0.9)	137.8 (3.1)	6.2	0.5	0.2	-88.9
C	98.5 (3.0)	113.6 (1.4)	131 (3.2)	9.3	0.1	0.2	-91.9
D	75.9 (2.7)	118.6 (0.5)	125.4 (0.9)	19.7	5.3	1.5	-41.9
E	22.43 (0.29)	53.33 (0.49)	71.77 (0.42)	47	0.07	11.43	-41.5
F	95.4 (1.9)	95.7 (1.1)	104.1 (1.0)	10.4	0.3	4	-58.7
G	57.73 (0.89)	41.23 (0.84)	101.67 (0.09)	29.87	10.67	6.60	-18.9

Note: Standard deviation was given in parentheses.

Table 3. Contact angles of water (θw), formamide (θF), diiodomethane (θD), Lifshitz-Van der Waals (γLW), electrons donor (γ–), electrons acceptor (γ+) parameters obtained with *S. aureus* ATCC25923 suspended in a solution of KNO_3 (0.1M).

Strain	Contact angles (°)			Surface tension: components and parameters ($mJ\ m^{-2}$)			ΔGiwi ($mJ\ m^{-2}$)
	θ diiomethane	θ formamide	θ water	γ (LW)	γ (+)	γ (-)	
S. aureus ATCC25923 0.1M	50.5 (2,9)	22.9 (1,0)	19.9 (2,6)	34	1.7	53	30.7

Note: Standard deviation was given in parentheses.

facial phenomena involved in the bacterial adhesion process allows limiting or even inhibiting this adhesion (8). Therefore, we had to calculate the total free energy of the adhesion process through LW, AB and electrostatic interactions. As the suspending liquid of the KNO_3 solution has a high ionic strength (0.1 M), the free energy of electrostatic interaction is neglected compared to the sum of ΔG^{LW} and ΔG^{AB} interactions (13, 14). The results obtained in Table 4 showed that the adhesion process is favorable (ΔG^{Tot}<0) for all masks but not with the same potential power. In addition, the results show that adhesion generally becomes more favorable because of the increase in $\Delta G^{Toal\ XDLVO}$. The adhesion potential of *S. aureus* ATCC25923 on the different masks allows to classify the masks as follows: mask D (ΔG^{Tot}= -15, 90 $mJ\ m^{-2}$) > mask E (ΔG^{Tot}= -12,57 $mJ\ m^{-2}$) >mask B (ΔG^{Tot}= -10 $mJ\ m^{-2}$) >mask C (ΔG^{Tot}= -9,58 $mJ\ m^{-2}$) > mask A (ΔG^{Tot}=-0,95mJ m^{-2}) >mask F (ΔG^{Tot}=0,60 $mJ\ m^{-2}$) > mask G (ΔG^{Tot}=3,62 $mJ\ m^{-2}$).

Scanning electron microscopy and energy dispersive X-ray analyzer. Results obtained in Fig. 1 show the whole structure of the fabric obtained by SEM and the quantitative value of the element constituting the surface of the masks obtained by EDX analyzer. The morphological structure of the masks is curled and rolled up to form bunches in the lateral and vertical directions. All the masks have an almost similar structure according to the images obtained by SEM except the mask D presents a different morphological structure compared to the other masks. In addition, all surfaces show the presence of two chemical elements, which are oxygen and carbon with different percentages. For masks (A, B, C, D, E, F, and G) the mass percentage of carbon was (68,84%; 62,18%; 60,87%; 61,82%; 56,38%; 64,67% and 57,50%) respectively. The mass percentage for oxygen was (31,16%; 37,82%; 39,22%; 38,18%; 43,62%; 35,33% and 42,50%) respectively.

Oxygen is the minor element in all surfaces while carbon is the major one. This chemical evaluation could help us to understand the attractive or repulsive behavior of *S. aureus* ATCC25923 on the surface of the facemasks.

Table 4. Lifshitz-Van der Waals ΔG^{LW} (mJ m^{-2}), acid–base ΔG^{AB} (mJ m^{-2}) and total interactions free energy ΔG^{Tot} (mJ m^{-2}) of adhesion between *S. aureus* ATCC25923 and the seven facemasks.

Masks	Lifshitz-Van der Waals ΔG^{LW} (mJ m^{-2})	acid–base ΔG^{AB} (mJ m^{-2})	Interaction free energy ΔG^{Tot} (mJ m^{-2})
A	4,61	-5,56	-0,95
B	5,11	-15,11	-10,00
C	3,78	-13,37	-9,58
D	0,49	-16,40	-15,90
E	-1,94	-10,63	-12,57
F	3,37	-2,77	0,60
G	-5,23	8,84	3,62

DISCUSSION

The use of the facemasks has known very large use in response to the Sars-Cov-2 outbreak (20). The surface geometry, the capillary geometry of textile fabric and the amount and the nature of the test liquid are the factors affecting the fiber/liquid interaction (21). In this study, we attempt to evaluate the physicochemical properties as a factor that several authors have suggested as affecting mask performance criteria (10). As a result, the evaluation of all these properties could be important to understand the potential of *S. aureus* ATCC25923 to adhere to the surface of these masks using the XDLVO approach.

In our investigation, all fabrics demonstrate a hydrophobic character. This character is probably due to the high amount of carbon constituting the surface of the facemask. In addition, the hydrophobicity of the masks differ between the seven mask. This variation is probably due to the difference of the mass percentage of each chemical element or to their distribution in the surface of these masks. Many works have reported that the surface hydrophobicity reduced significantly with the simultaneous reduction in nitrogen concentration (22). In addition, cell surface hydrophobicity is modulated significantly under the conditions of carbon and nitrogen in growth media (23). In the literature, it is already reported that the wettability and interfacial characterization of natural fiber are affected by certain properties namely chemical and physical properties (24, 25). The composition of the fibers differs between natural and synthetic fibers, the naturel fiber is composed mainly of cellulose (88.0-96.5%), protein (1.0-1.9%), wax (0.4-1.2%), pectin (0.4-1.2%), inorganics (0.7-1.6%), and other (0.6-8.0%) (21), while polyester fiber (synthetic) is a blend of ethylene glycol and terephthalic acid. The hydrophobic character of the masks used in our study is similar to the hydrophobicity of other textile fibers. Indeed, kenaf fibers have higher contact angles of 87.24° with lower total surface energy (26). In addition, it was found that the wettability of Polyamide was 86° and that of Polyester was 87.5° (27). Besides, a research study show that the wettability of polyethylene terephthalate was 72° (28), and according to a recent study, the wettability of cellulose brut fiber extracted from *Spartium junceum* L. was 99° (29).

Through this work, we have investigated other surface properties such as the polar acid-base proprieties using contact angle method. The acid-base properties are important for the adhesion phenomenon (6, 8, 30) Besides, the important role of acid base properties in microbial adhesion is already demonstrated by several studies (6, 8). In addition, acid–base interactions play the most important role in bacterial attachment to surfaces (31). In our investigation, it appears that facemasks, which have a high value of electron donor parameter, are those, which have a high percentage of oxygen. This result agrees with a study which demonstrate that acid base forces are governed by functional group exposed on the surface namely the phosphoric, carboxylic and amine groups (32). Besides, for *Escherichia coli* surfaces a correlation was found between the electron donor parameter and the P/C ratio (7).

Using the XDLVO approach to predict microbial adhesion on different materials such as stainless steel (31) and cedar wood surfaces (15) was previously documented and widely discussed by several research studies (15, 33, 34). The XDLVO approach is a theory that allows the quantification of the adhesion potential of a bacterium on a surface by the calculation of $\Delta G^{Total\ XDLVO}$. Bacterial adhesion on a substrate is a processes linked to the factor responsible of this

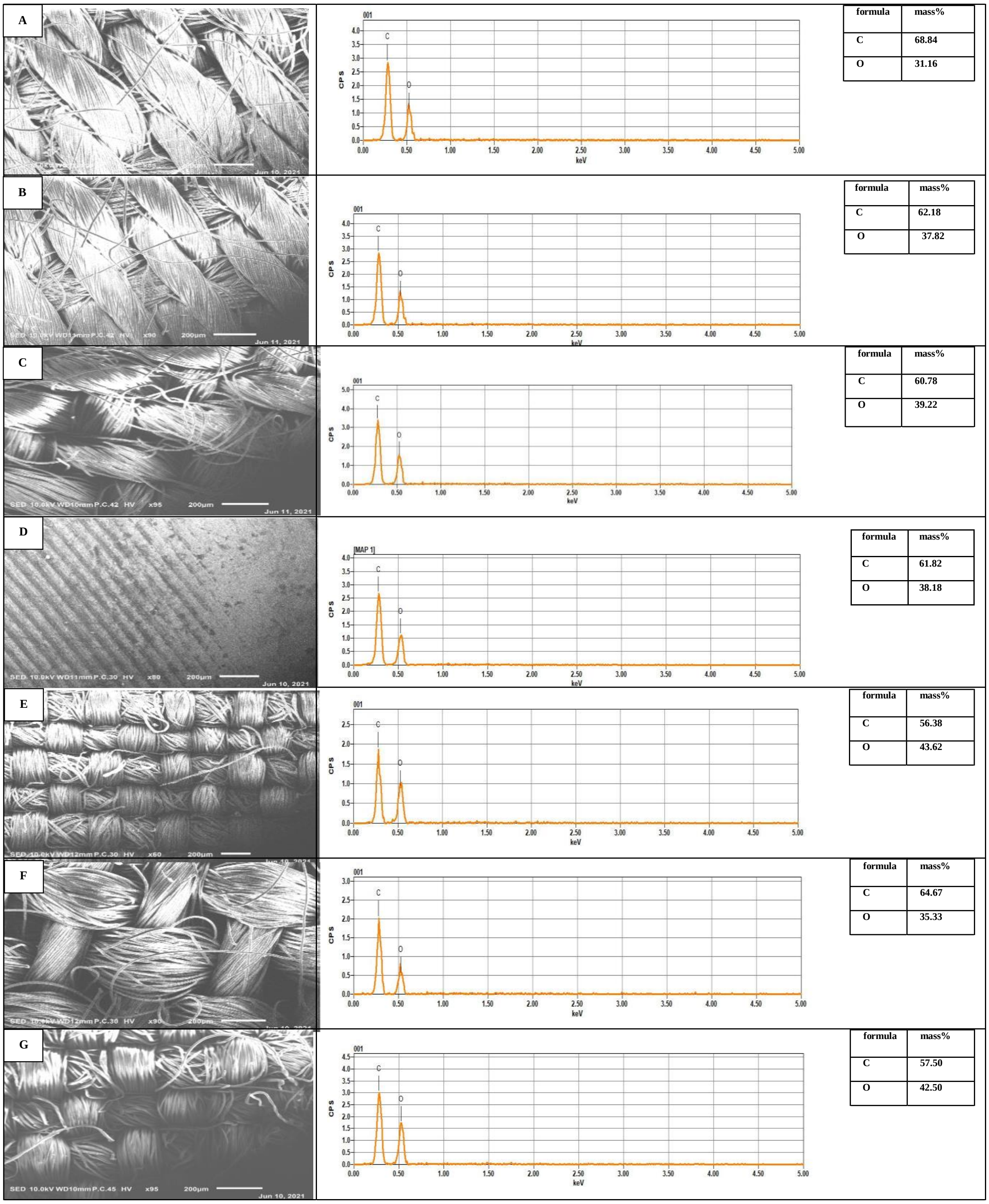

Fig. 1. SEM images of seven masks and EDX analysis

adhesion, namely the Lifshitz-Van der Waals (LW), Lewis acid base (AB) and the hydrophobicity (33).

Pertinent works have shown that the interaction between microorganisms and abiotic surfaces is of capital importance in environmental systems (35). In addition, a high value of ΔG^{Tot} can be explained by the attractive interaction between a textile and the surface of bacteria. To our knowledge, adhesion of *S. aureus* on facemask surfaces has not been investigated in literature. The prediction of adhesion is estimated by the value of ΔG^{Tot}, when it is negative, the adhesion is favorable. In contrast a positive value of ΔG^{Tot} predict unfavorable adhesion (8).

Our results show that the theoretical adhesion is favorable on masks used but the adhesion strength was different between masks. In addition, *S. aureus* ATCC25923 do not show the same behavior toward the mask (A) and the mask (D) despite the fact that they have the same hydrophobicity (ϴwater=125°). In this case, the hydrophobicity does not play a role in the adhesion phenomena, which show the intervention of the other surface interactions. Besides, high values of ΔG^{Tot} are related to high values of ΔG^{AB} and low values of ΔG^{LW}. Consequently, we speculate that theoretical adhesion of *S. aureus* ATCC25923 on masks is probably governed by acid base interaction. Our result is in agreement with the speculation of another works when investigating the adhesion of *S. aureus* ATCC25923 on Teflon (8). It is reported that reusable masks are minimally protective compared to surgical masks. The risk of contamination by these masks becomes more important because of moisture and liquid retention of virus (36). Moreover, one study that evaluated the use of cloth masks in a health care facility found that health care workers using cotton cloth masks have increased risk of influenza-like illness compared with those who use surgical masks (37).

CONCLUSION

In literature, no study has reported the adhesion of *S. aureus* ATCC25923 on the surface of reusable masks using XDLVO approach. Our results revealed that the seven masks surfaces are hydrophobic. The electron donor/acceptor parameter varies between the seven facemasks. The behavior of *S. aureus* ATCC25923 was not the same between all masks used. The variation of surface properties between the different facemasks was affected the adhesion of *S. aureus* ATCC25923 and consequently it could be a factor influencing the performance criteria of these masks.

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