Universidade do Minho
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Physico-chemical interactions between fibre-based wiping materials and disinfectant affecting antimicrobial efficacy

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STATEMENT OF INTEGRITY

I hereby declare having conducted this academic work with integrity. I confirm that I have not used plagiarism or any form of undue use of information or falsification of results along the process leading to its elaboration.

I further declare that I have fully acknowledged the Code of Ethical Conduct of the University of Minho.

Resumo

O uso de toalhetes à base de fibras impregnados com desinfetantes são o método mais comum para a desinfeção de superfícies em contexto hospitalar, indústrias de processamento e aplicações domésticas. Este trabalho estuda a interação entre o desinfetante cloreto de alquildimetilbenzilamónio (ADBAC) e toalhetes comerciais compostos por três materiais distintos, W1 – 100% poliéster, W2 – 55% celulose/45% poliéster e W3 – 100% celulose, a sua eficácia antimicrobiana e o efeito do tratamento com descarga plasmática de barreira dielétrica (DBD).

Demonstrou-se que a adsorção de ADBAC é significativamente influenciada pela composição do substrato têxtil em termos de teor de celulose, razão de banho e tempo de imersão. Os substratos com maior teor de celulose apresentam maior adsorção de ADBAC. No entanto, na análise por espectroscopia de raio-X (XPS), o substrato de poliéster foi o que apresentou a maior concentração de ADBAC na superfície. Os resultados de XPS também demonstraram a geração de novas espécies de oxigénio na sua superfície após o tratamento DBD, o que aumentou de forma substancial a concentração de ADBAC. Na celulose, o plasma apenas promoveu um efeito de erosão. No estudo de envelhecimento não se observaram diferenças significativas na força e alongamento à rotura durante o armazenamento de W1 e W2. No entanto, a força à rotura no sentido da trama do substrato W3 tratado com plasma diminuiu. Os resultados das análises mecânicas dinâmicas demonstraram que a imersão em ADBAC e o tratamento DBD tiveram uma influência significativa nas propriedades viscoelásticas de W1, pelo melhoramento da resposta elástica devido à diminuição da mobilidade das cadeias poliméricas, e de W3, pelo melhoramento da resposta não elástica devido à erosão plasmática. O substrato W1 foi o que demonstrou maior eficácia antimicrobiana, sendo este efeito mais evidenciado e mantido ao longo do tempo nas amostras tratadas com plasma. Por outro lado, o W2 não tratado com plasma exibiu maior efeito antimicrobiano em bactérias Gram-positivas, mas com o tratamento DBD, a atividade antimicrobiana foi maior nas Gram-negativas. O substrato W3 apresenta baixa atividade antimicrobiana, confirmando os resultados apresentados na literatura para a celulose.

Em geral, este projeto permitiu o desenvolvimento de uma nova classe de toalhetes à base de poliéster de custo acessível e ambientalmente sustentável. Estes possuem uma eficácia antimicrobiana superior, melhorada pelo tratamento DBD, devido à maior concentração de ADBAC na superfície do toalhete, podendo ser armazenados por período de tempo mais longos.

Palavras-chave: compostos quaternários de amónia; desinfeção de superfícies; plasma de descarga de barreira dielétrica (DBD); toalhetes.

Abstract

Surface disinfection by disinfectant-impregnated wipes is the most prevalent disinfection method used in nosocomial environment, food processing industry and other domestic situations. This work studies the interaction between the alkyldimethylbenzylammonium chloride (ADBAC) disinfectant and three untreated and dielectric barrier discharge (DBD) plasma-treated commercial wiping materials of W1 – 100% polyester, W2 – 55% cellulose/45% polyester and W3 – 100% cellulose (cotton) affecting the antimicrobial efficacy.

Wipe material type in terms of cellulose content, liquor ratio and immersion time demonstrated a significant influence on the adsorption of ADBAC in both untreated and plasma-treated samples. The higher the content of cellulose in the material, the higher is the adsorption of ADBAC active ingredient. Nevertheless, X-ray photoelectron spectroscopy (XPS) analysis found higher ADBAC concentration on the surface of polyester wipe than the other two. Also, it confirmed the interaction of the ADBAC with the newly generated oxygen species on the polyester surface when plasma treatment was applied, drastically increasing the ADBAC concentration on the surface. Whereas, plasma treatment only resulted in etching effects on cellulose. In the ageing study, no significant changes in breaking force and elongation during storage for untreated and plasma-treated W1 and W2 were observed. However, plasma treatment affects W3 in weft direction reducing the force at break. Dynamic mechanical analysis results showed that ADBAC immersion and plasma treatment have a significant influence in viscoelastic properties of W1, by improving its elastic response limiting the polymeric chains mobility and of W3, by enhancing the non-elastic response due to the etching effect. W1 displayed the highest antimicrobial efficacy, with more enhanced and prolonged performance in plasma-treated samples. W2 showed high antimicrobial effect but plasma treatment caused an inversion in its performance against Gram-positive and Gram-negative bacteria. W3 exhibited low antimicrobial activity, confirming the negative result presented in literature.

Overall, plasma treatment allows a new class of low-cost and environmental-friendly polyester-based wiping materials with superior antimicrobial efficacy due to improved ADBAC concentration on the wipe surface, which is maintained during a longer storage period.

Keywords: dielectric barrier discharge (DBD) plasma; quaternary ammonium compounds; surface disinfection; wipes.

Table of Content

Acronyms and notations

A

Abs	Absorbance
Abs_0	Absorbance of the ADBAC stock solution
Abs_t	Absorbance of the ADBAC solution after removing the wipe
ADBAC	Alkyl-dimethyl-benzyl-ammonium chloride
AHP	Aerosolized hydrogen peroxide system
AISI	American iron and steel institute
ANOVA	Analysis of variance
AOAC	Association of Official Agricultural Chemists
APPJ	Atmospheric pressure jet
Ar	Argon
ARD	Automatic room disinfection
ASTM	American Society for Testing and Materials
ATCC	American Type Culture Collection
ATP	Adenosine triphosphate
ATR	Total reflectance accessory

B

BCG	*Mycobacterium bovis*
B. thuringiensis	*Bacillus thuringiensis*
BPD	Biocidal Products Directive

C

C-C	Carbon-carbon bond
C-H	Carbon-hydrogen bond
C-N	Carbon-nitrogen bond
C-O	Carbon-oxygen single bond
C=O	Carbonyl
C_0	Initial ADBAC stock concentration

CA	Cellulose acetate
cm	Centimetre
CAWP	Commercially available wipe product
CD	Cross direction
CDC	Centres for Disease Control and Prevention
CEN	European Committee for Normalization
CFU	Colony forming unit
CO_2	Carbon dioxide
COO	Carboxyl
C_Q	Concentration of ADBAC in the wipe
C_R	Concentration reduction of ADBAC in the bulk solution
C_t	Concentration of the ADBAC after removing the wipe
CV	Coefficient of variance

D

DBD	Dielectric barrier discharge
DCA	Dynamic contact angle
DIWS	Disinfectant-impregnated wipe system
DIWs	Disinfectant-impregnated wipes
DMA	Dynamic Mechanical Analysis
DNA	Deoxyribonucleic acid
DSC	Differential Scanning Calorimetry
DW	Distilled water

E

E’	Storage modulus
E’’	Loss modulus
ECHA	European chemicals agency
E. coli	*Escherichia coli*
EDANA	The European Disposables and Nonwovens Association
EPA	United States Environmental Protection Agency
EU	European Union

eV	Electron Volt

F

FTIR	Fourier transform infrared spectroscopy
FWHM	Full Wave at Half Maximum

G

g	Gram
G1	First generation
GPa	Gigapascal
GSTs	Germicidal spray tests

H

H_2O_2	Hydrogen peroxide
HCAIs	Healthcare - associated infections
HDPE	High-density polyethylene
HICPAC	Healthcare Infection Control Practices Advisory Committee
HPV	Hydrogen peroxide vapour system

I

ICU	Intensive care unit
INDA	Association of the Nonwoven Fabrics Industry
IPC	Infection Prevention and Control
ISO	International organization for standardization

L

l	Width of treatment
L	Litre
Log R	Microorganism log 10 reduction

K

kHz	Kilohertz

kV	kilovolt
kW	Kilowatt

M

m	Meter
MD	Machine direction
mg	Milligram
min(s)	Minute(s)
mJ	Millijoule
mm	Millimetre
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSSA	Methicillin-susceptible *Staphylococcus aureus*
M_w	Initial weight of the wipe sample

N

n	Number of passages
N	Newton
n.a.	Not available
nm	Nanometre
N_2	Nitrogen
NCBI	National Center for Biotechnology Information
NMMO	N-Methylmorpholine N-oxide

O

O2	Oxygen
OH	Hydroxyl
OPA	O-phthaldialdehyde
OPP	Office of Pesticide Programs

P

p	Plasma power
P	Plasma-treated wipe sample

PAA	Peracetic acid
P. aeruginosa	*Pseudomonas aeruginosa*
PCL	Polycaprolactone
PEG	Polyethylene glycol 200
PET	Polyethylene terephthalate
PHB	Polyhydroxybutyrate
PHBV	Polyhydroxybutyrate co valerate
PIDW	Pre-impregnated disinfecting wipe (pre-wetted disinfecting wipe)
PLA	Polylactic acid
ppm	Part per million
PSDW	Pre-soaked disinfecting wipe (bucket method)
PUR	Polyurethane
PV-1	Poliovirus 1
PVC	Polyvinyl chloride

Q

Q_0	ADBAC amount in stock solution
QACs	Quaternary ammonium compounds
QPM	Petri plate method
Q_t	ADBAC amount remained in solution after removing the wipe
Q_w	ADBAC amount absorbed in the wipe sample

R

R	Control (untreated) wipe sample
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
RH	Relative humidity
RKI	German Federal Robert Koch Institute
RNA	Ribonucleic acid
rpm	Rotations per minute
RTUDW	“Ready-to-use” disinfecting wipes
R_w	Weight ratio between the amount of ADBAC on the wipe and the wipe mass

S

S_0	The initial total weight (Tube + ADBAC solution)
Sa	Arithmetical mean height
S. aureus	*Staphylococcus aureus*
SCA	Stationary contact angle
SD	Standard deviation
Sdr	Developed interfacial area ratio
sec/s	Second(s)
Spc	Arithmetic mean peak curvature
S_t	Total weight after removing the wipe (Tube + ADBAC solution - Wipe)
S_w	Initial total weight with immersed wipe (Tube + ADBAC solution + Wipe)
Sz	Maximum height

T

tan δ	Damping factor
TC 216	Technical Committee 216
TSA	Tryptone soya agar
TSB	Tryptic Soy Broth

U

UTM	Universal testing machine
UV	Ultraviolet

V

v	Velocity
V_0	Volume of the initial ADBAC bulk solution
Vis	Visible
V_w	Volume of the solution absorbed by the wipe sample

W

W	Wipe
W_0	Weight of the Initial ADBAC solution

Wa	Warp
WAdh	Work of adhesion
We	Weft
W_t	Weight of the ADBAC solution after removing the wipe
W_w	Weight of the solution absorbed by the wipe sample

X

XPS	X-ray photoelectron spectroscopy

Others

°C	Celsius degree
α	Significant level in ANOVA
γ	Surface free energy
γ^D	Dispersion forces
γ^l	Liquid surface free energy
γ^P	Polar forces
θ	Contact angle
λ_{max}	Wavelength at the peak
μ	Micro-

Index of Figures

Index of Tables

Index of Figures in Annex

Index of Tables in Annex

Acknowledgements 2

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Publications and Communications

Papers in Journal

- **PAPER: Song X,** Cvelbar U, Strazar P, Vossebein L, Zille A. Antimicrobial Efficiency and Surface Interactions of Quaternary Ammonium Compound Absorbed on Dielectric Barrier Discharge (DBD) Plasma Treated Fiber-Based Wiping Materials. ACS Applied Materials and Interfaces, 2019; 12. **DOI: 10.1021/acsami.9b18746**

- **PAPER**: **Song X**, Cvelbar U, Strazar P, Vossebein L, Zille A. Chemical, thermo-mechanical and antimicrobial properties of DBD plasma-treated disinfectant-impregnated wipes during storage. Polymers 2019; 11. **DOI: 10.3390/polym11111769**

- **PAPER**: **Song X**, Vossebein L, Zille A. Efficacy of disinfectant-impregnated wipes used for surface disinfection in hospitals: a review. Antimicrobial Resistance & Infection Control 2019; 8. **DOI: 10.1186/s13756-019-0595-2**

- **PAPER Conference**: **Song X**, Vossebein L, Zille A. 2018. Structure properties change of ready-to-use nonwoven wiping materials over storage time. IOP Conference Series: Materials Science and Engineering. 460:012055. **DOI: 10.1088/1757-899X/460/1/012055**

Book chapter

- **Xinyu Song**, Jorge Padrão, Ana Isabel Ribeiro, Andrea Zille. Invited Book chapter "Testing, characterization and regulations of antimicrobial textiles" in Book "Antimicrobial Textiles from Natural Resources".

Posters and abstract in conference

- **POSTER: Song X**, Vossebein L, Zille A. "Storage time effects on the bactericidal activity of plasma-treated quats disinfectant-impregnated wipes". 5th International Conference on Prevention and Infection Control 2019", 10-13 September, 2019, Geneva, Switzerland. **DOI: 10.6084/m9.figshare.9852293**

- **POSTER**: **Song X**, Vossebein L, Zille A. "Structure properties change of ready-to-use nonwoven wiping materials over storage time". 18th World Textile Conference "AUTEX 2018", 20-22 June, 2018, Istanbul, Turkey. **DOI: 10.6084/m9.figshare.9971489**

- **ABSTRACT**: **Song X**, Vossebein L, Zille A. Storage time effects on the bactericidal activity of plasma-treated quats disinfectant-impregnated wipes. P252, Abstracts from the 5th International Conference on Prevention & Infection Control (ICPIC 2019). Antimicrobial Resistance & Infection Control 2019; 8. **DOI: 10.1186/s13756-019-0567-6**

Chapter 1

Introduction

1. INTRODUCTION

1.1. Background

Healthcare-associated infections (or hospital-acquired infections) prevention and control are nationally and internationally emphasized for the safety of public health. There are in general two sources for the cause of healthcare-associated infections (HCAIs): medical devices associated infections and environment associated infections. HCAIs caused by the transfer of nosocomial pathogens from high-touch environmental surfaces (e.g. door handles, bedrails, call buttons, toilet seats, as well as surfaces in intensive care units (ICU) and surgical sites) and medical devices are responsible for significant patient's morbidity, mortality and economic cost [1-3]. More recent evidence shows nosocomial pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA), norovirus, *Clostridium difficile*, *vancomycin-resistant Enterococcus*, *Acinetobacter* species and coronavirus etc. shed by patients can contaminate hospital surfaces at concentrations sufficient for transmission, surviving for extended periods and persisting despite attempts to remove them [4-6]. Cross-contamination has been reported not only in nosocomial environment but also in the food processing industry and other domestic situations [7].

An effective cleaning and disinfection practice plays a key role in preventing cross-contamination and spread of HCAIs [8-11]. Failure of cleaning and disinfection could cause severe outbreaks of infection transmission in hospitals, substantially disturbing the clinical workflow [12]. There are many approaches for surface disinfection, for instance, simply by heat, ultraviolet germicidal irradiation, automatic room disinfection (ARD) by hydrogen peroxide vapour system (HPV) or aerosolized hydrogen peroxide system (AHP) [13]. Increased development of advanced novel disinfection strategies, such as self-disinfecting surfaces with the integration of antimicrobial agents on the surface, are gaining attention in their application in clinical settings [14, 15]. However, results from clinical trials suggested antimicrobial coated surfaces as a complementary approach but cannot be the substitution of routine cleaning and disinfection for infection prevention and control (IPC) in hospitals [16-18]. The utilization of chemical disinfectant is broadly diffused in food industry, hospitals and healthcare centres because of its effective cost performance, easy application and relatively broad antimicrobial spectrum [13, 19-22]. Disinfectants are based on a wide range of active ingredients such as alcohols (isopropanol; ethanol, 2-

butoxyethanol), chlorine and chlorine compounds (hypochlorites, chlorine dioxide, and Chloramine-T), aldehydes (formaldehyde, glutaraldehyde, ortho-phthalaldehyde), hydrogen peroxide, iodophors, phenolics, peracetic acid and quaternary ammonium compounds (alkyl dimethyl ammonium chloride, dodecyl dimethyl ammonium chloride, and alkyl dimethyl ethylbenzyl ammonium chloride). Among all, quaternary ammonium compounds (QACs or quats) are vastly utilized in surface disinfection because of its thermal stability, low human toxicity and oxidative properties, low cost and adequate germicidal performance [23, 24]. The effectiveness of disinfectants is generally tested by assessing the mechanisms of action of the active substance, solution stability, dosage and its interaction with the target organism [25-27].

In disinfection practice, traditionally, healthcare staff has been using the "Bucket method" (known as well as pre-soaked disinfecting wipe), which consists in towels saturated with diluted disinfectant solution contained in a bucket or an equal container. However, the "bucket method" exhibits several limitations such as improper dilution, inappropriate material (e.g. cellulose material, microfiber), double-dipping, reusing after contamination, and inadequate saturation time [25, 28-31]. On one hand, when more cleaning cloths are submerged in the same bucket, reduction of disinfectant concentration in the bucket by taking out the cloth is often neglected [32]. On the other hand, fear of infections promotes the use of a high amount of disinfectant without considering toxicity (e.g. occupational asthma among cleaning personnel while using disinfectants like formaldehyde, glutaraldehyde and chorine) or cost benefit [33, 34]. Other studies show that some hospitals utilising disinfectant dispensing stations, distribute disinfectant solutions at a concentration that greatly differs from expected levels [28, 35]. Nevertheless, because of its relatively low cost and simple implementation, "bucket methods" is still in use for disinfection in some hospitals.

The "ready-to-use" disinfecting wipes (RTUDW) (also reported as pre-impregnated disinfecting wipes, pre-saturated towelette and pre-wetted disinfecting wipe in some literature) are increasingly accepted and broadly diffused for decontamination of high-touch surfaces because of their convenient implementation in practice and reliable performance [36-38]. Moreover, the visible debris and organic load that could hinder the disinfectant effectiveness can be easily removed by textile wipes [39, 40]. Back then to 1988, one laboratory investigated the disposable and reusable disinfectant cloths for cleaning food contact surfaces. The study suggested that the use of paper or disposable wipes can reduce the risk of cross-contamination [41]. Many studies regarding the efficacy of disinfecting wipes

validated the benefits of the application of disinfecting wipes in terms of disinfection performance and working compliance [39, 40, 42, 43].

In the disinfectant-impregnated wipe systems (including both pre-impregnated and pre-soaked disinfecting wipes), one essential element is the wipe, known as well as towelette, wiper, and cloth etc. Most wipes are based on papers or nonwoven textiles made from cotton, wood pulp, viscose, lyocell, polyester and polypropylene. Although disinfecting wipes have been widely used for disinfection purpose [44, 45], adsorption of disinfectant due to the presence of certain textile materials has been previously reported in the literature [35, 46, 47]. The use of an inappropriate wipe material (e.g. cellulosic material) could interact with the absorbed active ingredient (e.g. QACs) resulting in lowering, or even inhibiting, the disinfectant efficacy [47]. Possible factors that influence the adsorption are the combination of wiping material and active ingredients, concentration, formulation of the disinfectant solution, temperature, pH value, immersion time, and target surface [48, 49].

Countable research has quantified disinfecting wipes' overall decontamination efficacy through assessing their ability to remove and prevent the microbial transfer between surfaces [50-53]. Little is known about the interaction of the textile substrates with disinfectants and its impact on disinfecting wipes' overall decontamination efficacy [46, 48, 54]. Studies in the past usually focus either only on one type of material or on the mechanical properties or exhibited poor experimental designs. Regarding the physicochemical interactions, the binding of QACs with cellulose-based textile materials is more pronounced and has been frequently reported so far [46-48, 55]. For example, Boyce et al. studied wipes with different soaking time submerged in the same bucket of QACs solution. However, the decrease of QACs concentration in the bucket due to the QACs adsorption on wipe was not taken into consideration [35]. Additionally, research from Hinchliffe et al. shows that the adsorption of QACs on the substrate was progressive by increasing the ratio of fabric to solution [48]. However, it was not possible to understand the impact of QACs binding to the wipe materials on their antimicrobial activity because of the lack of proper microbiology tests. Likewise, research from Bloss et al. did not conduct any microbiology test [47]. Another research from Engelbrecht et al. with a similar experimental design has performed the microbiology tests [46]. However, these tests did not consider bacterial removal due to the mechanical wiping action and the adhesion of the bacteria on the fibres [54]. Currently, there are no systematic studies that provide solid evidence to scientifically explain the interaction mechanism between the disinfectant active ingredients and wiping materials as well as the impact of the interaction on the overall decontamination activity of the disinfecting wipes. Moreover, it remains unknown

regarding the ageing performance of disinfectant-impregnated wipes in storage. In polymer material, five types of ageing classifications exist, physical ageing, photochemical degradation, thermal degradation, chemical attack, and mechanical stress [56]. For professional use (industry, hospitals, healthcare centres, etc.), the disinfectant-impregnated wipes have to be stored in a shaded, cool, dry, stable and well-ventilated place. Thus, photochemical, thermal and mechanical degradations usually have a low impact and physical ageing occurs all along the lifetime of the product [56, 57]. However, to the best knowledge of the authors, the understandings of the storage chemical ageing of wipes in the presence of disinfectant are very poor. Chemical attack on textiles due to disinfectants immersion can change the material properties from soft to hard and stiff or lowering the lint levels [58]. Furthermore, the interaction between wipe material and disinfectant during storage can also affect the products' disinfection performance.

In the last years, concerns about the fate of QACs in the environment have emerged due to their toxicity to a wide range of aquatic organisms and the rise of antibiotic resistance [59]. In an effort to avoid excessive use or abuse of QACs and minimize QACs unnecessary spread in the environment, the proper application and use of the disinfectant to is of paramount importance [60]. There is an apparent need for improving the hospital environmental surface hygiene regime and reducing the HACIs.

1.2. Objective and Research Questions

The main objective of this PhD project is to study the physicochemical interaction mechanism between different wiping materials and the active ingredient in QACs and how this interaction influences the system's overall antimicrobial efficacy. The new insights into interaction mechanisms among wiping materials and QACs benefits for long-term exploitation of efficient, reliable and cost-competitive disinfectant-impregnated wipes, contributes to the infection prevention and control (IPC) in hospitals.

Three research questions are brought forward from the project:

i. What is the physicochemical interaction mechanism/adsorption kinetics between wiping material and active ingredients in pre-impregnated disinfecting wipe? Taking into account the factors - material type, immersion time, and liquor ratio (fabric to liquid).

ii. How does the interaction influence the system's (pre-impregnated disinfecting wipe) overall decontamination effectiveness, encountering with various types of microorganisms (Gram-positive or Gram-negative bacteria)?

iii. How do the disinfectant-impregnated wipes perform over storage time? Considering the change of structure, function, and chemical and thermo-mechanical properties of wiping materials, adsorption of active ingredients onto textile substrate, as well as the antimicrobial efficacy.

As specific objectives, the project aims at developing:

- A comprehensive selection of commercial and in-house prepared wipes to study a wide range of different fibre compositions and textile structures coping with a fully morphological, physicochemical and thermo-mechanical characterization of the selected wiping materials in terms of bulk and surface analysis, wettability, air permeability etc.
- The chemical characterization and application of selected disinfectant on the wipes. Study the kinetics of absorption and adsorption between the disinfectant and textile substrate in different materials composition, immersion times and liquor ratios.
- Evaluation of structure, chemical and thermo-mechanical properties, and antimicrobial efficacy change of disinfectant-impregnated wipes during storage (Ageing performance).
- Antimicrobial tests performed for i) the assessment of the impact of interaction between the wiping material and disinfectant on disinfecting wipe's antimicrobial efficacy; ii) the evaluation of the antimicrobial efficacy of the eluate from the disinfecting wipes; iii) simulation of disinfectant-impregnated wipes used in practice with Phase 2 step 2 microbiology test.
- Also, surface chemistry modification in a Dielectric Barrier Discharge (DBD) atmospheric plasma treatment were applied to the selected wipe samples to positively improve the interaction among QACs disinfectant and wipe materials. All the tests mentioned above were performed with plasma-treated wipe samples as well.

1.3. Outline of the thesis

The Thesis is developed in the following chapters.

Chapter 1 Introduction

Chapter 1 gave a general introduction and background of the thesis topic and brought out the objective and research questions of the PhD project.

Chapter 2 State of the art

A systematic literature review based on the five categories i. wipes, ii. disinfectants, iii. application methods, iv. interaction between wipes and active ingredients and v. wiping strategy which can possibly influence the disinfection effectiveness of DIWs was conducted by Google scholar. Studies regarding the efficacy evaluation of DIWs in clinical applications were also reviewed from the National Center for Biotechnology Information (NCBI) database. Efficacy test protocols, standards were reviewed and summarized. The employed DBD plasma treatment was introduced as well. The literature review result was critically discussed as well. Chapter 2 is based on the published review paper:

Song X, Vossebein L, Zille A, Efficacy of disinfectant-impregnated wipes used for surface disinfection in hospitals: a review. Antimicrobial Resistance & Infection Control 2019; 8. DOI: 10.1186/s13756-019-0595-2.

Chapter 3 Materials and methods

Chapter 3 listed detailed information of the materials - disinfectant and wiping materials, used in the investigation, as well as all the methods and techniques applied in the development of the PhD project.

Chapter 4 Results and discussion

Chapter 4 presented and discussed the main results obtained in the project and was structured into four sections following the main tasks presented.

1. Absorption and adsorption
2. Chemical interaction analysis
3. Ageing performance
4. Antimicrobial efficacy tests

Chapter 4 is based on two empirical papers:

Song X, Cvelbar U, Strazar P, Vossebein L, Zille A. Antimicrobial Efficiency and Surface Interactions of Quaternary Ammonium Compound Absorbed on Dielectric Barrier Discharge (DBD) Plasma Treated

Fiber-Based Wiping Materials. ACS Applied Materials and Interfaces, 2019; 12. DOI: 10.1021/acsami.9b18746

Song X, Cvelbar U, Strazar P, Vossebein L, Zille A. Chemical, thermo-mechanical and antimicrobial properties of DBD plasma-treated disinfectant-impregnated wipes during storage. Polymers 2019; 11. DOI: 10.3390/polym11111769

Chapter 5 Conclusion and future research

Chapter 5 summarized the discussion from chapter 4 and presented the overall conclusion of the thesis, as well as proposed future research directions.

Chapter 2

State of the art

2. STATE OF THE ART

The use of pre-impregnated disinfecting wipes is one of the most efficient and prevalent methods for the decontamination of high-touch environmental surfaces and non-critical medical devices in hospitals, healthcare centres, and food processing industries. Despite this, the effectiveness of disinfecting wipes in the decontamination process is always in discussion. Disinfectant-impregnated wipes (DIWs) basically consists of towels saturated with diluted disinfectant as well as other chemical products such as surfactants, preservatives, enzymes, and perfumes etc. When two materials encounter, the interaction between each other is not negligible and often has influences on their original function.

Several parameters influencing the antimicrobial efficacy embracing the external factors for instance target surface (material, organic load), target microorganism, ambient environment (temperature, humidity), and internal factors such as the disinfectant (type, concentration), wipe (material type, construction/fibre architecture), application method, wiping strategy, are investigated by numerous researchers. A systematic literature search focusing on the internal factors were conducted by Google scholar. General efficacy study of DIWs in clinical practice was searched on NCBI (National Center for Biotechnology Information) database. For additional information related to the efficacy testing protocols, standards were explored and reviewed under the scope of EU standards issued by the European Committee for Normalization (CEN), Technical Committee 216 (TC 216) and US standards by the United States Environmental Protection Agency (EPA) Office of Pesticide Programs (OPP) in cooperation with Association of Official Agricultural Chemists (AOAC) International and American Society for Testing and Materials (ASTM) International. Besides, guidelines i.e. Guidance on the Biocidal Products Regulation from European Chemicals Agency (ECHA) and Guideline for Disinfection and Sterilization in Healthcare Facilities" by Healthcare Infection Control Practices Advisory Committee (HICPAC) of Centres for Disease Control and Prevention of USA (CDC) are also included for the literature review.

The state of the art is structured in the aspect of wipes, disinfectants, application methods, interaction between wipes and active ingredients, wiping strategies, factors that could possibly influence the system's disinfection efficacy; exhibition of the efficacy study of DIW in literature; an evaluation of the standards testing antimicrobial efficacy of pre-impregnated disinfecting wipes as well as the introduction of plasma technology at the end. Critical discussion regarding the literature review is presented at the end.

2.1. Wipes

Wipes according to its end-use are designed to absorb, retain or release dust or liquids [61, 62]. Wipes are generally used for cleaning purpose, in conjunction with other substances, performing different functions like polishing, cleaning and disinfection [63, 64]. The wet wipes are the most growing market in the last years consisting of mainly personal care, household/home cleaning, and industrial cleaning wipes. They are used in numerous application areas such as face and eye cleaning, makeup removing, sun protection, self-tanning, antiperspirant, insect-repellent lotion applications on the skin, treatment of dry or oily skin, body cleaning, and surface cleaning in houses and industry [65]. The principal focus of this project was given to the wipes used for disinfection purpose.

The wipe, known as well as towelette, cloth, or wiper can be made of various materials, e.g. textile substrate or paper-based. Textile-based wipes can be structured by nonwoven, weaving or knitting techniques, but because of its superior cost-effectiveness and good performance, the nonwoven is the most common manufacture method (especially for disposable wipes) [66]. However, it is important to distinguish paper- from textile-based structures, specifically wet-laid paper from wet-laid nonwovens [66]. Paper-based wipes are manufactured from the pulp of cellulose fibres, generally, wood pulp held together by hydrogen bonds [67]. Different materials and bonding systems between nonwoven and paper production could perform differently in contact with active ingredients in the disinfectant solution.

In the international acknowledged definition from ISO (International organization for standardization) Standard 9092:2011 and CEN EN 29092 (also known as EN ISO 9092:2011), it is clearly stated that film and paper structures are not considered as nonwovens. The definition of nonwoven proposed by two associations of global leading roles in Nonwoven industry –The European Disposables and Nonwovens Association (EDANA) and Association of the Nonwoven Fabrics Industry (INDA) specify that wet-laid webs are considered nonwovens if *"they contain a minimum of 50% of man-made fibres or other fibres of non-vegetable origin with a length to diameter ratio equals or superior to 300, or a minimum of 30% of man-made fibres with a length to diameter ratio equals or superior to 600, and a maximum apparent density of 0.40 g/cm^3"*.

There are other classifications of wipes based on different perspectives. Considering end-user applications, wipes are subdivided into two categories: consumer wipes and industrial wipes [68]. Consumer wipes include personal care wipes, i.e. adult wipes, baby wipes, cosmetic wipes such as facial cleaning and deodorant wipes, and household cleaning wipes. Industrial wipes are mainly related

with the wipes used in manufacturing, engineering and maintenance in the automotive, transportation, printing, food industry, janitorial, electronic, computer and optical industries, but also in hospitals for polishing, cleaning and disinfection functions [69]. On the other hand, considering the wipes final preparation, they are classified as dry and wet wipes [70]. Dry wipes are mainly for household use and are pure textile products without containing any visible liquid after their manufacturing. Wet wipes, or more precisely pre-wetted wipes, are most demanded in personal care and industrial uses for cleaning, polishing and disinfection and are imbibed with different solutions for each specific use. Usually, they can contain detergents composed of various surfactants, preservatives, enzymes, and perfumes or other products according to their final purposes. According to the lifespan of the wipes, they can also be classified as disposable and durable wipes, which can also be described as single-use wipes and reusable wipes. Durable wipes can be reused after a valid procedure such as standard industry washing process, but often face the problem of function deficiency after laundry. Disposable wipes mean that the wipes have a single or limited use and become a waste material after use, which in turn can be recycled, composted, incinerated or disposed of in a landfill [71]. Nowadays, disposable wipes have strong penetration in the market because they provide low risks of cross-contamination and high cleaning efficiency. Moreover, the recent development in non-woven technology leads to a significant reduction of production costs giving additional support for the growth of the disposable wipes' diffusion in the market [72].

Regarding the disinfecting wipes, wipes offer a cleaning procedure by the mechanical action of wiping, which can remove the organic debris along with the disinfection activity. Likewise, the microorganisms can be mechanically removed by the wipe. However, attention should be paid to the transfer of microorganisms to other parts of the surface. The removal of the microorganisms depends on the inherent properties of the wiping material such as surface energy, fabric structure and fibre types as well as by the applied pressure force, the geometry of the mechanical action, the number of passages and type of microorganisms adhesion mechanism [54, 73]. As stated before, it is also important to consider that during the wiping action some microorganisms could be just transferred in another place of the treated surface instead of being removed. This transfer depends by the wipe retaining ability and by the bactericidal activity of the disinfectant adsorbed into the wipe [54].

The disinfectant solution released by the wipe on the target surface is mainly responsible for the bactericidal activity. The quantity and concentration of active ingredient and the amount of the solution remaining on the surface are important efficacy indicators and depending on the interaction between

the wipe and disinfectant. Also, the amount of released solution is highly dependent on the wipe absorbent property. Without any doubt, wipe plays an important role in decontamination of the target surface. A comprehensive literature review regarding the material, production, and application of the wipes in the market are presented below.

2.1.1 Advanced wipes in the market

The most important cutting-edge technologies for wipes in the market were introduced in the following.

Microfiber and nanofiber wipes

Microfiber wipe relates to wipe made from fibres whose diameter is in the range of microscale. Likewise, nanofiber wipes refer to that the diameter of its raw material fibres in the range of nanoscale. However, in the fibre industry, there is no universally accepted definition of nanofiber or microfiber. There are different opinions regarding the diameter range of nanofibers i.e. smaller than 500 nanometres [74], under 100 nanometres [75], or less than 10 nanometres [76]. Also, microfibers have different definitions such as fibres with diameters ranging from 0.5 to 5 micrometres [74], with diameter from 2 to 10 micrometres [77], or fibres with a diameter of 0.3 micrometres [78]. Spunbonding and meltblowing extrusions can produce bicomponent microfiber with special techniques such as the “islands in the sea” [74, 79]. More detailed information of spunbonding and meltblowing is given in the section of wipe production. The “islands in the sea” refers to the fibrils of one polymer dispersed in a matrix of another polymer. The fibrils are the islands and the matrix is the sea. Eventually, the matrix polymer can be dissolved, and the fibrils of the other polymers are split in the size of microfiber, Therefore, it is possible to reduce the diameter of the fibrils increasing the number of islands in the cross-section area of the sea [80]. Electrospinning is regarding as the most used technique for producing fibres with the diameter ranging from several micrometres down to 100 nm or less [81]. The setup and production process are relatively simple and inexpensive. It is a fibre spinning method with high voltage electrical field accelerating polymeric solutions [76, 78, 81-83]. The morphologies of the fibres can be easily modified and controlled by varying electrospinning conditions [84]. In combination with the hydroentangling bonding, it is a prevalent way to produce micro-denier fabric [85]. More detailed information regarding electrospinning technology and hydroentangling technique is given in the section of wipe production. Microfiber wipes are relatively new in the market of the surface disinfection but their cleansing and disinfection efficiency have been evaluated by numerous

studies [86-88]. Some demonstrated that microfiber system has superior microbial removal efficiency compared with cotton string mops [89, 90]. Others stated that the use of microfiber cloth spread the bacteria although there was an overall reduction in bacterial counts on the contaminated surface [91].

Composite wipes

Composite nonwovens wipes are composed of a mixture of fibres and particulates or of fibres that differ in their chemistry, count number or shape to provide improved functionality at lower cost [70]. Composite nonwovens give a wide range of different functionalities to wipes using a variety of combinations among nonwoven production techniques, layering and fibre mixtures. One classic example is the meltblown and/or spunbond nonwoven wipe laminated on the surface with cotton fibres in the core layer. The outer meltblown or spunbond web made of polypropylene serves as a binder in the thermal bonding process and assist the liquid acquisition and transport to the highly absorbent cotton core [92]. The advantage of composite wipes is their good durability maintaining at the same time good absorbency properties.

Biodegradable wipes

Biodegradable wipes are of great interest for their obvious environmental and sustainability advantages. For years the use of natural fibres like cotton, flax, jute, kenaf, etc. was the priority choice. However, several new biodegradable fibres including regenerated cellulosic materials such as cellulose acetate (CA), rayon and lyocell, and synthetic and bio-based polymers such as polylactic acid (PLA), polycaprolactone (PCL), polyhydroxybutyrate (PHB) and polyhydroxybutyrate co valerate (PHBV) have gained the attention of the nonwoven industry [69, 93]. The nonwoven fabrics are usually composed of cotton fibres thermal bonded using bio-based thermoplastic polymers providing the soft and absorbent property of cotton alongside the increased strength provided by the synthetic biodegradable fibres [93].

Flushable wipes

Flushable nonwoven wipes are emerging and evolving since 2004. The leading global nonwovens associations INDA and EDANA have recently reached a consensus on the definition of flushability, publishing since 2008 the "Guidelines Document for Assessing the Flushability of Nonwoven Disposable Products". Flushable nonwoven wipes are designed to be able to be flushed down the wastewater system without adversely impacting plumbing or wastewater infrastructure and operations

[94]. Regarding the definition of a flushable nonwoven wipe, all the material used must be biodegradable and without any chemicals that might affect the functioning of the sewage farm or the quality of the treated water [69]. Triggerable latex bonded air-laid, Hydraspun™ composites, and conventional wet-laid, air-laid and spunlace technology are the currently available manufacturing technologies for flushable nonwoven wipes. Polyvinyl acetate/polyvinyl alcohol, wood pulp, and lyocell have been reported for the production of flushable nonwoven wipes [95, 96]. The technical difficulty of flushable wipes is that the wipe must break down immediately in a toilet bowl and be small enough to be transported from the toilet bowl to the sewage system in a single flush without causing clogging, blockages or equipment failure in the wastewater conveyance and treatment systems but at the same time it has to maintain strong enough to be stored and used when wet. In one word, one must make a balance between the dispersion ability, strength, and biodegradability [97]. Due to the concern of environmental protection and sustainable development, the flushable nonwoven wipe is a technology with great potential for future development and it is strongly supported by the industry. However, the presence of polyethylene terephthalate (PET), high-density polyethylene (HDPE) and other plastic fibres was observed in several brands flushable wipes raising some concerns about the absence of national and international regulations on wet wipes labelling/advertising regarding flushability and disposal [98].
The market for disposable nonwovens has vastly increased recently with all their advantages mentioned above, which also increase the problem related to their landfill management as waste [99]. For the concern of environment protection and sustainable development, the flushable biodegradable nonwoven wipes are strongly recommended by the industry. Table 1 below looks at available advanced wipes.

Table 1. Advanced wipes in the market and their advantages and disadvantages.

Advanced wipes	Description	Advantages	Disadvantages	Ref.
Microfiber wipes	Microfiber wipe relates to wipe made from fibres whose diameter is in the range of micro scale.	Its cleaning and disinfection efficiency have been evaluated by numerous studies. Some demonstrated that microfiber system has superior microbial removal efficiency compared with cotton string mops.	Others stated that the use of microfiber cloth spread the bacteria although there was an overall reduction in bacterial counts on the contaminated surface.	[86-88, 91]
Composite wipes	Composite nonwovens wipes are composed of a mixture of fibres and particulates or of fibres that differ in their chemistry, count number or shape in order to provide improved functionality at lower cost.	The advantage of composite wipes is their good durability maintaining at the same time good absorbency properties.	Different materials composition may limit the production process choice.	[86-88, 91]
Biodegradable wipes	The nonwoven fabrics are usually composed of cotton fibres thermal bonded using bio-based thermoplastic polymers.	Providing the soft and absorbent property from cotton alongside the increased strength by the synthetic biodegradable fibres. Biodegradable wipes are of great interest for their obvious environmental and sustainability advantages.	More cost in the aspect of material	[93, 94]
Flushable wipes	Flushable nonwoven wipes are designed to be able to be flushed down the wastewater system without adversely impacting plumbing or wastewater infrastructure and operations.	A relief to landfill management as waste in the concern of environment protection and sustainable development. Flushable nonwoven is strongly supported by the industry.	There is technical difficulty with flushable wipes: the wipe must break down immediately in a toilet bowl and be small enough to be transported from the toilet bowl to the sewage system in a single flush without causing clogging, blockages or equipment failure in the wastewater conveyance and treatment systems but at the same time it has to maintain strong enough to be stored and used when wet.	[93]

2.1.2 Wipe production

Searching from the literature and wipe products existing in the market, it is quite straightforward to conclude that the majority of wipes are made by nonwoven processes embracing numerous wipe products such as, wipes for hygiene use, pre-wetted wipe for cosmetic tissue, cleaning towel, polishing cloths, demisting cloths, antistatic cloths, electrostatic dedusting cloths etc. [66, 100, 101]. For the wipes used for disinfection purpose, high liquid absorbency, good mechanical stability and flexibility are required. For high water absorbency property, the wipe should not only be capable of large absorption of the target solution but also ensure a sufficient amount of liquid to be released on the surface. Regarding the mechanical stability especially the wet strength, the wipe should exhibit good tear resistance, low elongation and good abrasion resistance allowing even heavy dirt removal without fibre shedding or breaking and size deformation in the cleaning process.

To achieve these properties in the wipe, several kinds of nonwoven processes can be applied [66]. There are generally two steps in the nonwoven process, web formation and bonding. Afterwards, if applicable, different finishing methods can be adopted to add more values on the wipe products. In the web-forming step, there are different approaches to implement such as dry-laid process (including carding and air-laid) and wet-laid process (Table 2). In the carding method viscose rayon, Tencel (formerly lyocell), cotton, polypropylene and polyester fibres with long cut lengths are normally used. Before carding, there can be the opening and blending stages [101]; In the air-laid system, fibres are dispersed in the air to make a very dilute volumetric suspension of short length fibres approximately between 15 mm to 60 mm [102]. Air-laid nonwoven products have the advantage of isotropic, lofty, high porosity, high absorbency and wicking rate, softer handle, good resiliency, adequate tensile strength and low cost. Wet-laid technology originally comes from the papermaking process. It has the capability to process a wide range of fibres including very short fibres (woodpulp) and brittle fibres. Man-made fibres used in the wet-laid method have to be cut approximately between 4mm to 20mm [102]. Wet-laid technology is a very important segment for flushable wipes' production [103].

Besides, there are also web-formed wipes produced without previous opening or carding, which refers to polymer-laid manufacture by extrusion technology such as spunbonding, meltblowing and electrospinning for nano- and micro-fibre production processes. Polymer-laid manufacturing has a very high production speed by eliminating intermediate steps in the nonwoven fabric production with considerable cost reduction [104]. Polymer-laid nonwovens play an important role in the application as oil sorbent wipes. However, it can only be used for thermoplastic fibres production. Spunbond

production starts with the extrusion of filaments followed by the attenuation and deposition of fibres. Spunbond method is typically used to produce the "islands in the sea" fibres [74]. Polyester, nylon (PA), polypropylene, polyethylene are conventionally used as island components and polystyrene, a copolymer of 2-ethylhexyl acrylate, ethylene terephthalate copolymer (COPET), as well as polylactic acid (PLA), are often used as the sea matrix [105]. Splitting of islands-in-the-sea fibres (e.g. PA6/COPET) can be processed during hydroentangling of nonwovens. Meltblown is a similar process like spunbond but produces much finer filaments and webs of excellent uniformity. The average fibre diameter is in between 1 and 5 micrometres. The most common used materials for meltblown are polypropylene [102].

Table 2. Web-forming techniques used in the production of nonwoven wipes.

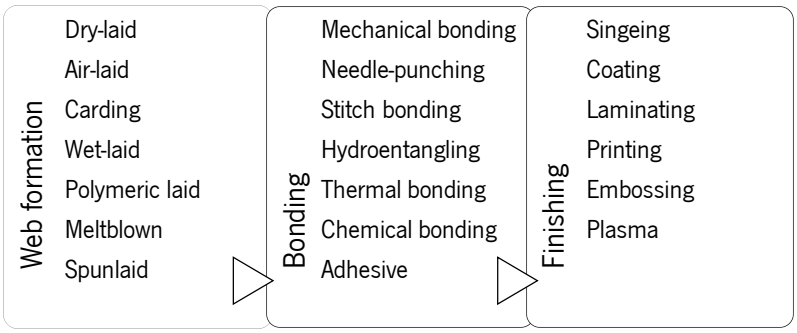
Web forming	Process material	Method	Application
Carding	Viscose rayon, lyocell, cotton, polypropylene and polyester fibres with long cut lengths	Rotating drum with metallic wires and teeth	Heavy duty industrial wipe and household wipes
Air-laid	Wide range of fibre including natural and synthetic thermoplastic fibres (fluff pulp)	Air flow	Disposable/single use wipes, flushable wipe and household cleaning wipes
Wet-laid	Cellulose pulp (woodpulp) and short cut length man-made fibres (viscose, polyester, nylon, polypropylene)	Water	Flushable wipe
Polymer-laid	Thermoplastic fibres e.g. polyester, polypropylene, nylon etc.	Extrusion technology	Oil sorbent wipes

Following the web formation is the bonding process. There are different bonding systems according to the raw materials and end products. There is mechanical bonding like needle punching, stitch punching and hydroentanglement (spunlacing), chemical bonding and thermal bonding. The needle-punching is the earliest developed process among all the others. The bonding of the fibre web is realised by interlocking the fibres through the web with barbered needles. It is also the only bonding method suitable for the production of heavyweight spunbond fabrics [106]. However, when they are produced by the needle-punching method with a fabric areal density below 100 g m^{-2}, the wipes lose their uniformity [104]. The needle-punched nonwoven is more often used in filtration application due to its

loftiness and distinctive porous structure [107]. Stitch bonding is a mechanical bonding method of consolidating the formed webs with knitting elements, which is more often used to produce home furnishings, geotextiles, vacuum bags but it is rarely used for wipe production [108]. Hydroentangling method intertwines the fibres by fine and high-pressure jets of water to accomplish the lock of the fibre web. It can be applied to manufacture all the natural fibres including cellulose fibre products. Hydroentangled wipe is well known for its high softness, drape, bulkiness and resilience [70]. Chemical bonding is a bonding process with the utilization of chemical binders, mainly latex. The chemically bonded wipes, depending on the chemicals used in the process, have low skin tolerance and are not environment-friendly. Thermal bonding is a method based on thermoplastic fibres, achieved by melting fibres or powder utilizing calendering or oven. This method cannot be used to produce wipes exclusively composed of natural fibres. Point bonding is a specific thermal bonding technique allowing substantial fibre mobility which contributes for the soft handling of the fabrics [104, 109]. Polypropylene is the most satisfactory fibre for thermal bonding method with a melting point of approximate 160 °C [109]. Thermal bonding is a cleaner, more energy saving and higher product quality technique comparing with the chemical bonding process [110].

The combination of web forming and bonding processes constitutes the essence of wipe production (Table 3 [111]). In a nonwoven production line, the web can be prepared using one or more separate web formation methods and later bonded with one or more bonding techniques [102]. Further finishing processes, for instance singeing, coating, laminating, printing, embossing, and plasma can be applied to provide specifics surface properties such as antistatic, antimicrobials, higher wettability and flame retardant among others to add more values on the products [108].

Table 3. Nonwoven production process with different methods.



Air-laid process in combination with hydroentangling (spunlacing) is expansively used in the manufacture of disposable single-use wipes because the air-laid process has the possibility of handling short pulp fibres with good cost performance and at the same time, the use of hydroentanglement is able to produce nonwoven products without chemical additives or thermal bonding. Airlaid-spunlace nonwovens offer textile-like handle (softness and flexibility) and strength to wipes [108, 112]. Heavy-duty industrial and household wipes are generally produced through carding and needling approach [70].

2.1.3 Wipe materials

The materials used in wipes vary depending on the application. The wipe for disinfection is mostly made of textile materials, including, but are not limited to, cellulosic fibres (cotton, woodpulp, viscose, lyocell) and thermoplastic fibres (polyethylene terephthalate, polypropylene, and polyamide). Particularly for disposable wipes, the raw materials are normally inexpensive like cellulosic fibres and polyolefine fibres. Cellulosic fibres are used to ensure high water retention and storage capacities and polyolefine fibres are accountable for high tensile strength, abrasion and solvent resistance [70]. The majority of wipes for surface disinfection in the market are made of blends of polyester and viscose fibres/woodpulp [70, 92]. Furthermore, the wipes areal density is small, generally between 25 g m^{-2} and 75 g m^{-2} [101].

Cotton

Cotton is the most important constituent of natural fibre used in wipes production. The current global share of cotton in the nonwoven market is about 2% and the value is projected to grow in the future years [113]. With the advantages of biodegradability, superior wet strength, high levels of absorbency as well as being soft and with a quick-drying surface, cotton is particularly used for wipes [68]. However, the presence of impurities in raw cotton, which affects the production and the quality of the finished products, strongly limits its extensive use in nonwoven production [101, 114]. Despite that, among all the traditional natural fibres used in wipe product (such as jute, kenaf, flax, hemp, wood, etc.), cotton is considered to be the most expensive one [115].

Cotton fibres are commonly blended with other synthetic fibres such as polyester and polypropylene to balance the weak mechanical property in the wipe products. Response to the trend of biodegradability and sustainability, biodegradable manufactured fibres like cellulose acetate (CA) and Eastar-Bio® (a

biodegradable aliphatic-aromatic copolyester) fibres were chosen to be applied as the binder fibre in cotton-based biodegradable nonwoven products [93, 116, 117].

Lignocellulosic Pulp

Pulp is a lignocellulosic fibrous material resulting from chemical or mechanical treatment of various types of materials such as cellulose fibres from wood, fibre crops, textiles rags or waste paper [118]. The type of treatment (e.g. thermo-mechanical pulps, mechanical-obtained medium density fibre pulps and chemically-treated kraft pulps) varies depending on the type of raw material [119, 120]. Nowadays, 90% of pulp is originated from wood, which is termed wood pulp [118]. Wood pulp can be classified as softwood pulp (long but wide) and hardwood pulp (short but narrow) depending on the origin wood plants [118]. Wood pulp is extensively employed for disposable wipe production owing to its abundance in nature and bargain price apart from its high absorbency [121, 122]. It is frequently used in the absorbent wipe production in combination with polyolefin staple fibres [123]. Wood pulp composite spunlaced nonwovens are a prominent design in the application of flushable nonwoven wipe [99, 124]. Since the length of wood pulp fibre is frequently less than 4 mm (average fibre length of softwood pulp and hardwood pulp is respectively 3.3 mm and 1.0 mm), wet-laid and air-laid techniques are the most suitable approaches for the production of wood pulp composing nonwoven [102, 118].

Regenerated cellulose

Viscose, modal and lyocell are classic cellulose regenerated materials, which is also called cellulosic chemical fibres. Nowadays the world-famous lyocell fibre producer - TENCEL® brand, as a trading name nearly represents the lyocell fibres in the market. The difference between viscose, modal and lyocell is the producing methods [101]. Viscose and modal are made using a very similar process regenerating the cellulose fibres using a similar chemical (sodium hydroxide). However, modal fibres are stretched to increase molecular alignment after spinning to make the filaments stronger. Lyocell uses a different and non-toxic solvent N-Methylmorpholine N-oxide (NMMO) to extract the cellulose from the wood. The use of NMMO is also more environmentally friendly because it is easier to filter and re-use [125].

Despite viscose is not easily spunlaid or thermally bonded, it has been extensively used in a nonwoven production owing to the following advantages [126]:

- Low cost

- Ease of processing on all types of web-forming and bonding equipment;
- A wide variety of description, i.e., range of counts, range of lengths, range of finishes, crimped and non-crimped, bleached and unbleached;
- Biodegradable, which is a grand trend for future wipe production.

The viscose production process starts with the production of the cellulose pulps using various wood types through different delignification processes following by extrusion [127]. The structural features, orientation, and lateral order in the extrusion process can vary and impact differently the viscose physical properties including tenacity and elongation [128].

Because of its properties and absence of impurities, viscose is widely used as a substitute for cotton in the production of wipes. Although lyocell shares many properties with viscose, such as the high wet strength, it is not as widely used as viscose or cotton fibres in the production of wipes due to its more expensive production process.

Polyethylene terephthalate (PET)

Polyethylene terephthalate is the most common member of the polyester family of polymers better known in the textile industry just as "polyester". The application of polyester in textile is widely offering a variety of fibre cross-section shapes, count number, lengths and finishes due to its properties that can be readily altered in the production of the polymer and manufacture processes of textile fabrics. Polyester fibres have good tensile strength, high modulus, high toughness, good abrasion resistance, resilience, chemical resistance and can be processed using any of the main methods of nonwoven manufactures [129]. However, for wipe production, which generally requires high absorbency, hydrophobicity of polyester is the main drawback. Therefore, either applying finishing to increase polyester's absorption ability (i.e. plasma treatment) or blending with cellulose fibres, is often required during the manufacturing process [130, 131]. However, as the trend goes for biodegradable, some studies have been performed to search for substitute (e.g. flax or biodegradable polyester) in the wipe production [92].

Polypropylene

Polypropylene fibres account for 63% of all the fibres used for nonwoven production in the world [66]. Polypropylene belongs to the general category of polyolefin polymers. Its oleophilic nature makes the

nonwoven fabrics efficient in absorbing and retaining oil from oil-water mixtures [129]. The reasons of polypropylene being predominant in the nonwoven industry are the following [66]:

- Low density and specific gravity allowing the production of lightweight fabrics.
- Low glass transition and melting temperature, which is attractive for thermal bonding.
- Inherent hydrophobicity that can be modified using opportune treatments.
- Provides fabrics with good bulk and cover.
- Chemical stability.
- Biological degradation resistance (mildew, perspiration).
- Stain and soil release
- Good mechanical strength and abrasion resistance.

Polypropylene can be used in needle-punched nonwovens for heavy weighted products like floor covering and geotextile and can also be thermally bonded for lightweight disposable wipes and hygiene products.

Polyamide

The most common polyamide fibres are polyamide 6, known as Perlon, and polyamide 6.6, known as Nylon. Polyamide is normally applied in textile products in the form of filament since the production cost of staple fibres of polyamide is higher. Therefore, polyamide is only used in nonwoven production in case of high tear strength is required. The advantages of polyamide include high durability, relatively high glass transition and melting temperatures, high tensile and tear strengths, good elastic recovery and low static electric charge generation. Disadvantages include poor resistance to exposure to light and poor wet strength [92]. Besides, polyamide has a superb response to the atmospheric plasma treatment for surface wettability and surface energy improvement [132].

2.2. Disinfectants

2.2.1 Disinfectants category

Disinfectant as the main constituent for disinfection action has a crucial impact on the decontamination process. Disinfectants comprise a wide variety of active chemical agents (biocides). The active

ingredients found in the market are generally alcohols, chlorine, aldehyde, peroxygens, biguanide, and quaternary ammonium compounds [133]. The antimicrobial activity of disinfectants performs in two different ways: growth inhibition (e.g. bacteriostatic, fungistatic) and lethal action (sporicidal, bactericidal, fungicidal, and virucidal effects) [134]. In 2004, the "Recommendation for Hygiene Requirements for Cleaning and Disinfecting Surfaces" was published by the Commission for Hospital Hygiene and Infection Prevention at the German Federal Robert Koch Institute (RKI) [135]. There the importance of environmental surfaces disinfection has been addressed and the risk areas in hospitals were classified. Therein, different surface disinfection procedures are categorized, such as routine disinfection, terminal disinfection, disinfection in the event of outbreaks, disinfection in case of officially ordered decontaminations, and targeted disinfection of visibly contaminated surfaces. Hygienic procedures (hygiene plans) have also been recommended. Likewise, in 2008, the "Guideline for Disinfection and Sterilization in Healthcare Facilities" was published by Healthcare Infection Control Practices Advisory Committee (HICPAC) of Centres for Disease Control and Prevention of USA (CDC) [136]. In this guideline, background study and recommended disinfection and sterilization procedure have been described in detail. However, only the most commonly used chemical disinfectants for non-critical items in nosocomial environment are listed in the guideline. Non-critical items are those that come in contact with intact skin but not mucous membranes. They are alcohol, chlorine and chlorine compounds, formaldehyde, glutaraldehyde, hydrogen peroxide, iodophors, peracetic acid, phenolics, and quaternary ammonium compounds (short form: QACs or quats). Noncritical items can be also divided into noncritical patient care items and noncritical environmental surfaces. Non-critical environmental surfaces usually refer to high-touch environmental surfaces such as tables, lockers, mattresses and bedrails, commodes, examination couches, keyboards, especially in the intensive care unit (ICU), and low-risk medical equipment like stethoscopes, blood pressure cuffs, pulse oximeters, ventilators, manual ventilation bags drug trolleys, intravenous pumps, ultrasound instruments [137]. In any case, all the disinfectant products should be registered by EPA (United States Environmental Protection Agency) in the US and by REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) in Europe (to name only a few). Disinfectants are classified under the group of pesticide, in the category of antimicrobial pesticides by EPA. Equivalently, disinfectants fall into "Main Group 1: Disinfectants" within the scheme of Biocidal Products Directive (BPD) issued by ECHA (European chemicals agency). Rutala and co-workers have done a comprehensive study on the disinfectants and sterilant regarding their mode of action, microbicide activity, advantages and disadvantages in their

application. The information is well described in several of their articles including the "Guideline for Disinfection and Sterilization in Healthcare Facilities, 2008" [50, 138, 139]. Every type of disinfectant presents some advantages and disadvantages allowing, or not, its use in wipes.

A brief summary of various types of active ingredients for disinfectant-impregnated wipes application, their chemical formulas, pros and cons are presented in Table 4. It should be noted that many of the active ingredients could be used independently or in combination with each other.

Table 4. Active ingredients, chemical formulas, pros and cons of their application in disinfectant-impregnated wipes.

Disinfectant category	Example of active ingredients	Chemical formula	Advantages	Shortcomings	Ref.
Alcohol	Ethyl alcohol (Ethanol)	C_2H_6O	Rapid bactericidal effect. No bacteriostatic action. Relatively cheap and easy to obtain. Wet the surface easily.	Tend to swell and harden rubber and certain plastics. Not sporicidal. Inflammable. Poor inactivation effectiveness was reported for some virus. Lack of efficacy in the presence of organic debris. Metal corrosive. Difficult in ensuring certain contact time in an open system.	[64, 140-143]
	Isopropyl alcohol (Isopropanol)	C_3H_8O			
Chlorine and chlorine compounds	Hypochlorites	ClO^-	Most used chlorine disinfectants. Large bactericidal spectrum. No toxic residues. Not affected by water hardness. Inexpensive and fast mode of action.	Corrosive to metals (>500 ppm). Inactivated by organic matter. Irritating and burning for skin, eyes and mucous membranes. Discolour and bleach textiles. Toxic chlorine gas formation in contact with ammonia or acid.	[45, 144-146]
	Chlorine dioxide	ClO_2	Wide spectrum of biocidal activity. Efficient mycobactericidal activity in short contacts time. It provides prolonged bactericidal effect than chlorine due to its high retain of antimicrobial active ingredients.	Long-term use can damage the outer plastic coat of some insertion tubes.	
	Chloramine-t trihydrate	$C_7H_7ClNNaO_2S$	Chlorine retains longer which results in more prolonged bactericidal effect	Occupational asthma has been reported.	

Peroxygens	Hydrogen peroxide	H_2O_2	Satisfying germicidal activity including bacterial spores (with longer contact time). Environment friendly due to its fast degradation. Accelerated hydrogen peroxide was developed with widened material compatibility and application variability.	May have chemical irritation resembling pseudomembranous colitis	[147]
	Peracetic acid (PAA)	$C_2H_4O_3$	Rapid action against all microorganisms at low concentration. Reinforced removal of organic material without residue. Effective in the presence of organic matter. Sporicidal at low temperatures	Corrosive to copper, brass, bronze, plain steel, and galvanized iron. (corrosion decline by additives and pH modifications) Unstable, particularly when diluted.	
Quaternary ammonium compounds (QACs or quats)	Alkyl dimethyl benzyl ammonium chloride	$C_{22}H_{40}N^+$	The most commonly used disinfectant in ordinary environmental surfaces with broad spectra of biocidal activity (lipid, enveloped viruses). Sporostatic. Good cleaning and deodorization property. Incorporation of QA moieties into polymers presents effective antimicrobial effect against biofilm.	Numerous studies show the adsorption of QACs onto the cotton substrate wiping material, which could lead to the failure of disinfection process. Susceptible with high water hardness. Less effective with Gram-negative bacteria and non-enveloped viruses.	[48, 148-154]
	Benzyl dimethyl octyl ammonium Chloride	$C_{17}H_{30}ClN$			
	Didecyl dimethyl ammonium chloride	$C_{22}H_{48}ClN$			

2.2.2 Disinfectants mode of action

The study of the modes of action of disinfectants can be dated to the beginning of the twentieth century [155]. However, the mechanism is very complex requiring the comprehensive examination of the structure and function of the various microorganisms including prions, endospores, mycobacterium, non-lipid virus, vegetative cells, fungi, and lipid virus [156]. Moreover, the mechanism of antimicrobial action of disinfectants is multiple and numerous hypothesises are still under investigation to understand their mode of action in detail [157]. A summary of the experimental data of disinfectants' target action is described in Table 5.

Table 5. Disinfectants' mode of actions.

Disinfectant	Mode of action	Ref.
Alcohol	Membrane damage and rapid denaturation proteins and action on the cytoplasm and nucleus (acidic compound) are the possible antimicrobial mechanisms.	[157]
Chlorine compounds	The antimicrobial action could be a combination of the following reasons: 1, oxidation of sulfhydryl enzymes and amino acids; 2, ring chlorination of amino acids; 3, loss of intracellular contents; 4, decreased uptake of nutrients; 5, inhibition of protein synthesis; 6, decreased oxygen uptake; 7, oxidation of respiratory components; 8, decreased adenosine triphosphate production; 9, breaks in DNA; 10, depressed DNA synthesis.	[157]
Formaldehyde	Alkylating the amino, carboxyl, and sulfhydryl groups of proteins and ring nitrogen atoms of purine bases; Cross-linking with nucleic acid thereby inhibiting DNA synthesis.	[158]
Glutaraldehyde	The biocidal activity of glutaraldehyde results from its alkylation of sulfhydryl, hydroxyl, carboxyl, and amino groups of microorganisms, which alters RNA, DNA, and protein synthesis.	[159]
Hydrogen peroxide	Create destructive hydroxyl free radicals that can attack membrane lipids, DNA, and other essential cell components.	[160]
Peracetic acid	The possible action could be oxidation lead to denature proteins, disrupt the cell wall permeability, oxidize sulfhydryl, and sulphur bonds in proteins, enzymes, and other metabolites	[160, 161]
Quaternary ammonium compounds (QACs)	Physical disruption of the cytoplasmic membrane of bacteria, following immediate leakage of intracellular constituents and lipids of viruses (including inactivation of energy-producing enzymes and denaturation of essential cell proteins). Autolysis due to QACs' lethality is also reported in contributing to cell death in some study.	[149, 162]

2.3. Application methods

Several studies have shown that high-touch environmental surfaces and devices can serve as a route for transmission of pathogens [163-165]. However, proper disinfection protocols and application strategies are still in development. When applying the surface disinfectant on the target surface, the approaches can be generally divided into two groups: i) without mechanical action, e.g. total immersion and directly spraying, and ii) with mechanical action, e.g. spray & wiping, dipping & wiping, and soaking & wiping [32]. The main benefit of mechanical action is its ability to remove the organic debris that could hinder the disinfection action.

All application methods can be found in use in practice for different surfaces. “Total Immersion” method is a good method for smaller sized medical devices. It is important to consider the material compatibility to avoid severe ageing of the device material due to large exposure surface and long contact time to disinfectant. “Spray”, “Spray and Wipe” and “Dip and Wipe” is not recommended for surface disinfection in general due to many drawbacks previously listed. “Soak and Wipe” is still commonly used in hospitals for daily cleaning and disinfection of large high-touch environmental surfaces such as floors, tables, lockers, examination couches. However, in this method, a potential interaction between disinfectant solution and wiping material can decrease the decontamination efficacy. The most prominent method is ready-to-use disinfecting wipes. Considering the antimicrobial efficacy of commercial wipes is already qualified by required standards before released into the market, there is less possibility of disinfection failure with this method. Nevertheless, ageing of the products needs to be further investigated as well as other parameters (e.g. wiping area, wiping passage, etc.) during the wiping process should be clarified by the manufacturer on the package. The detailed information regarding each application method is listed below.

2.3.1 Total Immersion

“Total immersion” is the more common-used method for the sterilization of medical devices, which normally starts with a thorough cleaning of the devices to eliminate the influence of the organic debris attached on the devices (e.g. with appropriate effort, can be removed by physical scrubbing or wiping) [143]. Following the cleaning, the devices are completely immersed in the solution for a specific time and temperature [166]. The solution for immersion can be an enzymatic solution, alkaline cleaner,

peracetic acid, hydrogen peroxide, iodophor, and sodium hypochlorite, or phenol disinfectants [64, 167]. When applying this method, it is important to consider that the materials of medical devices (stainless steel, plastics, etc.) must be able to withstand harsh conditions, especially chemically resist to various disinfectant, and at the same time maintain their properties for the intended use [168, 169]. On the one hand, the direct contact of disinfectant with the target surface can bypass the issue related with the absorption of additional compounds, such as surfactants, in the chemical preparations as well as avoid the failure of the decontamination treatment due to the volatility property of some disinfectants such as alcohol and chloride dioxide [48, 170]. On the other hand, the "Total Immersion" method is limited to the dimension of the surface size and the compatibility of the surface material with disinfectant always needs to be considered. Relatively large contact area and long contact time can increase the risks of swelling and hardening rubber and corroding metal parts of the device [143, 171, 172].

2.3.2 Spray

This simple method consists of a direct spray of the disinfectant solution with an aerosol or trigger sprayer on the target surface. In the decontamination/disinfection of environmental surfaces in room-size area also exists a new automated room decontamination system based on chemical (H_2O_2) vapour or mist, such as aerosolized hydrogen peroxide (AHP) system, dry gas vaporized hydrogen peroxide system or micro-condensation hydrogen peroxide vapour (HPV) system [36]. Its action mechanism is similar to the spray method. However, this new system is intended to be used as a supplement for traditional cleaning and disinfection procedures instead of alternatives or replacement [173]. The "Spray" method gives the chance of the direct contact between the disinfectant and target surface. The direct contact prevents the absorption or adsorption of active ingredients by a third media such as wipe, rag or sponge [46]. However, there are several drawbacks such as possible overspray, difficulty in covering surfaces (undersides of bedrails), atomized disinfectant in the air can subsequently be breathed in by workers and patients [174], without cleaning ability of visible debris on the surface [40, 43] and unacceptable drying time for routine hospital use [43].

2.3.3 Spray and Wipe

The "Spray and Wipe" method starts with the same process as in the "Spray" method, followed by a wipe of the target surface. Because of the wiping process, this method has the ability to clean the

visible debris on the surface and at the same time allows direct contact of the disinfectant solution with the target surface. The "Spray and Wipe" method shares the same drawbacks of the "Spray" method [174]. It is important to note that, due to the flammability of numerous sprayed disinfectants, the presence of open fires during use have to be taken into account [175].

2.3.4 Dip and Wipe

"Dip and Wipe" means dipping a dry towelette into a disinfectant solution for 5-10 seconds, wring out the excess solution and directly use it for disinfecting hard surfaces. The dry towelette can be composed of various textile materials, like cotton, polyester, polyethylene etc. Also, in this case, due to the presence of a wiping process, there is the benefit of removing visible debris from the target surface. However, the short contact time that the wipe spends in the disinfectant solution can limit the concentration of active ingredients applied on the target surface. A towelette carrying an insufficient amount of surface disinfectant may lose its antimicrobial activity and later becomes itself a potential vehicle of pathogen transmission [176]. In addition, the inappropriate reuse of the towelette may promote the accumulation of microorganisms and raise the risk of cross-contamination during the disinfection process [30, 31, 41].

2.3.5 Soak and Wipe

The "Soak and Wipe" method, also known as the "bucket method", was widely used for disinfection processes in hospitals in the past. It is quite similar to the "Dip and Wipe" method [177, 178]. The main difference is the soaking time of the towelette in the disinfectant solution. The towelette is soaked into disinfectant solution from 10 minutes up to 8 hours, before wrung out the excess solution and directly applied to a hard surface. The "Soak and Wipe" method was the most prevailing methods among all above-mentioned ones, owing to its acceptable compliance and easy application. It allows the removal of the visible debris that could influence the disinfectant performance and a relatively long contact time ensuring a sufficient amount of active ingredient loads in the towelette before use. Nevertheless, there are some studies reporting possible interactions between wipes and disinfectant due to the longer soaking time resulting in reduced antimicrobial activity of disinfectant [35, 47, 48]. Moreover, a chemical binding of the disinfectant to the wipe could lead to a decrease of disinfectant concentration in the bulk solution [47]. As indicated in the aforementioned method, improper reuse of the towelette can result in cross-transmission of pathogens on the treated surfaces [30, 31, 41].

2.3.6 Ready-to-use Disinfecting Wipe

A ready-to-use disinfecting wipe (Abbreviated as RTUDW) is a pre-wetted towelette containing disinfectant, antiseptic, surfactant etc. in a sealed package ready to use for surface disinfection up to one month (shelf life can be longer to two years). The use of RTUDW is steady increasing partially profit from the rapid development in nonwoven technology, providing a relatively good cost performance [179]. The RTUDW is designed to be used without any preparation time. Considering the compliance, employee time, and costs, RTUDW is highly recommended for surface disinfection in hospitals [42]. It has been tested in many research projects to be proved to possess good antimicrobial effect in several conditions [41, 64, 180]. RTUDW also includes an important cleaning step. But most important, RTUDW is disposable, which eliminates the possible contamination and transfer of pathogen from towelettes reuse [41]. However, the longer storage time could increase the probability of losing antimicrobial activity due to the possible binding of active ingredients onto the towelettes or by the degradation of the active ingredient [29]. Moreover, disposable properties could be a problem in its waste management.

2.4. Interaction between wiping materials and disinfectants

As mentioned in the introduction part, the disinfectant-impregnated wipes are facing the problem of interaction between textile material and disinfectant. The disinfection process of a disinfectant-impregnated wipe system (DIWS) can be divided into two parts that constitute the overall decontamination activity. One part is related to the microorganisms taken away by the wipe itself by means of mechanical action. The other part is related to the active microbicide action of the disinfectant solution released by the wipe on the surface.

The microorganism mechanically removed by the wipe has been critically discussed in the section of Wipe. The disinfectant solution released by the wipe on the target surface is mainly responsible for the bactericidal activity and is a result of the interaction between the wipe and disinfectant. A few investigations have been performed evaluating the interaction between wiping materials and surface disinfectants. Unfortunately, nearly all of them were exclusively focusing on the interaction between quaternary ammonium salts (QACs) and cotton substrate. Bloss et al. (2010) have classified the absorption of active ingredients onto textile substrate by testing three different surface disinfectants and

four different types of fabrics. They found out that the exposure of diluted surface disinfectants to various types of fabrics resulted in considerable adsorption of active ingredients [47]. Additionally, Boyce et al. (2015) found that several factors, including the soaking time and quats binding to specific wiping material, influence the antimicrobial efficacy of quats-based disinfectants. However, their experimental design showed two severe limitations: i) the wipes were taken out for quats concentration test in chronological order and the adsorption of wipes accounting for the decrease of quats concentration in the bucket was not taken into consideration; and ii) the lack of microbiological tests can hardly determine whether the low concentrations of quats released from the three wiping materials resulted in less potent reduction of bacterial counts on surfaces [35]. Some research has also found a decreased adsorption of QACs with the increase of the polyester blend in the cotton-based nonwoven wipe [55]. The investigation of Hinchliffe et al. (2016) may be the first comprehensive study of the possible parameters from both perspectives of the textile substrate and disinfectant solution influencing the quats binding degree onto a cotton substrate. They found that the amount of alkyl-dimethyl-benzyl-ammonium chloride (ADBAC) depleted from solution varied with the liquor ratio, pH, temperature, concentration of electrolytes and type of pre-treatment applied to the textile substrate. However, their investigation only measured the adsorption of active ingredients onto textile substrate in the bulk solution instead of the loss of active ingredients during the application stage (resulting from the binding of the active ingredients on the textile substrate) [48]. Later, they demonstrate that quats adsorption onto cotton substrate can be minimized and maintain the efficacy against Gram-negative (*Pseudomonas aeruginosa*) and Gram-positive (*Staphylococcus aureus*) bacteria [181].

In summary, disinfectant concentration, material compatibility, contact time, liquor ratio (wipe mass/disinfectant solution volume), an additive of other chemicals, and temperature are possible parameters impacting on the interaction of disinfectant and wipes.

2.5. Wiping strategies

Several studies have shown that high-touched environmental surfaces and devices can serve as a route for transmission of pathogens [163-165]. However, proper disinfection protocols and wiping strategies are still in development.

Wiping strategy includes the applied pressure force, wiped surface area, the geometry of the mechanical action, the number of passages etc. One recent study from A. M. West et al. tested the bactericidal efficacy of ten ready-to-use disinfectants in the form of pre-wetted towelettes [182]. The objective of the study focusses on the impact of surface area(s) wiped on its bactericidal efficacy. The result implicates a larger wiping surface area may lead to decreased bactericidal efficacy. However, rare attention is given for this factor, especially a severe lack of consideration from the efficacy testing standards (Detailed discussion is given in the next section).

2.6. Standards for disinfecting wipes' efficacy test

It is clear that an internationally recognized method to guarantee the evaluation of disinfecting wipe's ability using quasi-realistic conditions, especially regarding test surfaces and cross-contamination, is urgently needed. Standards listed below can evaluate the overall antimicrobial efficacy of the testing wipes, but not differentiate the mechanical removal of inoculum from a surface and the chemical inactivation of the test organisms. Also, the wiping strategy should be addressed as one factor that can have a considerable impact on the disinfection efficacy of DIWs. Divergent outcomes with different test standards can be suspected [183]. A guideline for comparable results between various test standards is in demand. This section opened the discussion from introducing standards background to existent standards for DIWs efficacy tests.

2.6.1 Standards background

In the last decades, numerous regulations and standards have been issued by various organisations for testing the efficacy of the disinfectants. The standards cover the most important factors that influence the effectiveness of a disinfectant, such as the target microorganism (bactericides, mycobactericides, sporicides/sterilants, fungicides, tuberculocides and virucides), the target surface (tile, stainless steel, wall panels, glass, etc.) and the application strategy (liquid, with wipe or spray method). Many protocols have been designed to validate the disinfectant's efficacy at the concentration commonly used against a panel of clinically significant microorganisms on the surfaces most routinely disinfected.

In EU, the disinfectant efficacy test is regulated and issued by the European Committee for Normalization (CEN), Technical Committee 216 (TC 216) under the work program "Chemical

Disinfectants and Antiseptics" [184]. Two phases were developed for assessing the disinfectant effect: 1) Phase 1 is mainly suspension-based tests for the basic evaluation of disinfectant efficacy against different microorganisms, apart from mycobacteria, under clean conditions. It is applied to evaluate the bactericidal (EN 1040), sporicidal (EN 14347) and fungicidal (EN 1275) activity of chemical antiseptics and disinfectants when appropriate standards are not available. It is a minimum requirement for the assessment of basic biocidal activity under generic conditions (food, industrial, domestic and institutional, medical and veterinary areas). 2) Phase 2 is designed for evaluation of the bactericidal, sporicidal, fungicidal and virucidal, activity of chemical disinfectants applied individually in specific conditions such as food, industrial, domestic, institutional, medical and veterinary areas. In the scope of Phase 2, European Norm is divided into two steps. Step 1 is a suspension test while step 2 is a carrier-based test. Both as suspension-based tests, Phase 2, step 1 test is prior than Phase 1 not only because the application area is more specific indicated in the test but also because it introduces the dirty conditions in testing the performance of surface disinfectant with the involvement of organic debris (Phase 1 only tests clean conditions). The dirty condition can demonstrate if a product (surface disinfectant) reacts with other substances such as proteins. Unfortunately, the suspension-based test is far away from the disinfectant performance in real practice. Consequently, carrier-based tests were developed to fulfil the need for disinfectant efficacy evaluation to various surfaces (instruments, surfaces, etc.) under practice-oriented conditions. Notably, in the carrier-based test, there are standards used for non-porous surfaces and porous surfaces with and without mechanical action. In the case of disinfecting-impregnated wipes, it applies to the standards for hard porous surfaces with mechanical action. In conclusion, standard EN 16615 is the most suitable one.

In the US, the United States Environmental Protection Agency (EPA) Office of Pesticide Programs (OPP) has the responsibility for regulating antimicrobial products used for treat and decontamination inanimate surfaces. In 1998, they first published the Product Performance Test Guidelines, OPPTS 810.2100 Products for Use on Hard Surface – Basic Efficacy Data Requirements used for efficacy testing of disinfectants in collaboration with AOAC International. Lately, it is amended as OSCPP 810.2200: Disinfectants for Use on Hard Surfaces – Efficacy Data Recommendations in September 2012. EPA recommended the carrier tests and use-dilution tests for assessment of disinfectant effectiveness for medical use surface disinfection [185]. Up to date, in cooperation with AOAC International and ASTM International, antimicrobial testing methods & procedures are well documented and specified from EPA's microbiology laboratory for antimicrobial formulations in the form of liquid,

spray and towelette, against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella choleraesuis*, *Mycobacterium bovis* (BCG), *Clostridium difficile*, Trichophyton mentagrophytes, non-enveloped viruses (i.e. parvovirus, noroviruses) and *Pseudomonas aeruginosa* or *Staphylococcus aureus* biofilm. Detailed testing methods are discussed in the following paragraphs.

AOAC Use Dilution Test is a standard operating procedure requested by EPA for evaluating liquid and dilutable liquid disinfectants for hard surfaces. Different series were developed for different microorganism tests - 955.14 (*Salmonella enterica*), 955.15 (*Staphylococcus aureus*), and 964.02 (*Pseudomonas aeruginosa*). The AOAC Use-Dilution Test is a relatively facile method to operate. However, it cannot demonstrate the use of disinfectants in practical conditions. Other methods are also specified by US EPA such as the AOAC METHOD 965.12 Tuberculocidal Activity of Disinfectants, which is a modified version of AOAC Use- Dilution test method applied to justify tuberculocidal efficacy claims for disinfectants. Due to the slow growth rate of the test microorganism (60 days' incubation time plus an additional 30 days), the test is susceptible to contaminations. AOAC METHOD 955.17 Fungicidal Activity Method, which is designed to access the effectiveness of the disinfectant's fungicidal activity. In the test, the highest acceptable dilution to disinfect a surface that is contaminated with the fungi in the given contact time is determined. AOAC METHOD 966.04 Sporicidal Activity of Disinfectants is developed to substantiate the sporicidal efficacy of high-level disinfectant or sterilant. By enumerating the number of spores per carrier and the dried spores on the surface, the test is recognised as a more robust challenge for the rigour of the disinfectant. AOAC METHOD 960.09 Germicidal and Detergent Sanitizing Action of Disinfectants test method is used to validate the efficacy of food contact surface disinfectant/sanitizer with Gram-negative and Gram-positive bacteria. AOAC Germicidal Spray Product Test: 961.02 (Germicidal spray products as Disinfectants) is used to evaluate the efficacy of disinfectant with the spray method on hard, non-porous surfaces. It is a semi-quantitative method based on statistics of passing and far away from real-life usage (extreme excess disinfectant quantity per unit surface area). Yet, this method has been modified and used for efficacy assessment of pre-saturated disinfecting towelettes.

ASTM International is another important standards organization that develops the disinfectant efficacy tests. There are several methods published by ASTM for the effectiveness assessment in terms of different application strategies (liquid, wipes), application areas (e.g. food contact surfaces or environmental surfaces and non-porous or porous surfaces etc.) and target microorganisms (bacteria, fungi, mycobacteria, spores, biofilm, virus). They are mainly suspension or carrier-based test methods.

The highlights in ASTM standards is that it embraces several efficacy tests of pre-impregnated towelettes. For instance, ASTM E2362-15, a qualitative method (provide no quantitative reductions) by estimation of growth positive and negative to determine the effectiveness of pre-saturated or impregnated towelettes for hard surface disinfection. The listed materials (apparatus) for testing are easily accessible in a regular microbiology lab. It includes a large spectrum of testing organisms as well including mycobacteria. Similar to ASTM E2362-15 is the ASTM E2896 – 12, which determining the effectiveness of antimicrobial towelettes with a quantitative Petri plate method instead of a glass slide. Originally designed by Williams et al. in their three-steps protocol to determine the efficacy of disinfectant wipes on surfaces and later amended into standard ASTM E2967-15. In this standard, extra equipment named Wiperator is requested.

2.6.2 Antimicrobial efficacy test standards for DIWs

In above-mentioned published standards by different world recognised organisations, efficacy tests regarding wipe/towelette are very few and recent. These available quantitative test protocols are critically discussed below.

The **EN 16615:2015** is a quantitative test method for the evaluation of bactericidal and yeasticidal activity on non-porous surfaces with mechanical action employing wipes for the medical area. This method displays several advantages allowing the quantitative evaluation of the antimicrobial activity of disinfecting wipes. It is applied to simulate the practical use of disinfecting wipes and allows to detect the cross-contamination caused by the wiping activity. Moreover, it can be used to evaluate the compatibility between the active ingredients in the solution and the wipe materials. It allows a flexible contact time (from 1 to 60 mins), can be tested in both clean and dirty conditions and define the declaration of concentration and exposure time on the disinfectants' labels. Despite these advantages, this method also displays some drawbacks. The test is considered relatively time consuming, complex and is not possible to strictly control the applied mechanical action. Monotonic test surface (PVC with PUR surface coating) and the test wipe (if not specified by request), as well as the fixed disinfectant volume (16 ml, considered to be a large ratio between disinfectant quantity and surface), could significantly influence the outcome. Moreover, it is difficult to discriminate between the microbicide activity derived from the disinfectant action (which represents the material compatibility issue) and the substrate that could retain microorganism by mere mechanical action.

The **Modified AOAC international method 961.02** is meant for the disinfection evaluation performance of pre-saturated towelettes for hard surfaces. It is a simple method to study the variables that could influence the disinfection outcome. Approved by EPA as a method for the registration of spray disinfectants, this method gives a straightforward picture of test products' performance providing survivor results in the form of a qualitative endpoint (growth positive versus growth negative). However, it exhibits unrealistic results when applied with a large ratio between disinfectant quantity and surface area. Besides, wiping applied pressure cannot be controlled, the concentration of bacteria on the test surface is not standardised, only allows semi-quantitative analysis, can only be applied in a monotonic surface (glass) and it is not possible to evaluate possible cross-contamination. Finally, because it does not address the humidity levels during the drying process of the test surfaces the results can be significantly uncertain.

The **ASTM E2896-12** is a quantitative standard test method meant for the determination of the effectiveness of antimicrobial towelettes. The listed materials (apparatus) for testing the wipes are easily accessible in a regular microbiology lab and it requires easy operational procedures to evaluate the disinfecting-wipe ability using glass Petri dishes and corkscrew pattern wiping movements. Simple modifications can be done to test other microbial strains. Despite that, this method presents some disadvantages such as the lack of control of several variables regarding the disinfectant-impregnated wipe (disinfectant amount, wipe size, etc.), its exclusive use in monotonic surfaces (e.g. glass Petri dishes), the impossibility to evaluate cross-contamination and the uncontrolled wiping action, especially the wiping pressure. Another drawback deserved to be mentioned is the inability to differentiating between mechanical removal of inoculum from a surface and chemical inactivation of the test microbe.

The **ASTM E2967-15** is a standard test method for assessing the ability of pre-wetted towelettes to remove and transfer bacterial contamination on hard, non-porous environmental surfaces using a specially designed machine to simulate the wiping action, the Wiperator. It allows great precision and reproducibility due to the well-controlled wiping action using the Wiperator. Despite, this test is not widely recognised in Europe, it fills some gaps existing in other evaluation methods allowing a realistic contact time and a quantitative evaluation of the antimicrobial activity of disinfecting wipe. Moreover, it guarantees the evaluation of the disinfecting wipe's ability to remove and prevent the microbial transfer from surfaces and their overall antimicrobial activity. However, in this case, the use of a monotonic test surface (stainless steel) and limited contact time (from 5 secs up to 45 secs) can restraint a realistic

outcome. There are some critics and debates related to the need for specific extra equipment (Wiperator) and whether the Wiperator could represent a realistic wiping process.

2.7. Efficacy test of disinfecting wipes in literature

Recently, the number of studies about the effectiveness of disinfecting wipes is steadily growing. Several parameters influencing the antimicrobial efficacy are investigated such as the type of disinfectant, wiping material, the combination of wipe and disinfectant, disinfectant concentration, target surface, target microorganism, immersion time, mechanical action, application strategy, etc. However, the combination of wipe and disinfectant, also known as material compatibility, is rarely addressed.

A countable number of studies regarding the efficacy of DIWs have been carried out. Tebbutt et al. may be the first ones, in 1988, to compare the decontamination performance of disposable and reusable disinfectant wipes concluding that the use of disposable disinfectant wipes significantly reduces the risks of cross-contamination. Moreover, their investigation was a breakthrough as it introduced the microbiological assessment of disinfecting wipes efficacy in practical use. The study not only examined whether the wipe transferred bacteria from one surface to another but also if any organisms remaining on the wipe has been killed [41]. Later, in 1993, Threlkeld et al. compared the disinfecting wipe method with the disinfectant soaking method in their efficacy to eliminate adenovirus 8 from medical instruments. The result revealed that the disinfectant wipe method could readily and thoroughly wipe away the virus from a tonometer and it was more convenient than disinfectant soaking method [64]. However, their finding cannot be safely extrapolated to other equipment items, which implies different target surfaces and organic load that may have an impact on the decontamination performance of disinfecting wipes. In 2007, Williams et al. developed a three-step protocol to quantify the efficacy of disinfectant wipes, their ability to remove and prevent the microbial transfer between surfaces and their overall antimicrobial activity, which could be considered as a milestone for the development of efficacy test for disinfecting wipes [73]. The paper introduced the first stringent test that is able to assess the ability of antimicrobial wipes to remove, kill and prevent the transfer of bacteria from contaminated surfaces. However, only one wiping material was used in this study, therefore no information can be extrapolated to understand the influence of different wiping materials in the surface disinfection efficacy with disinfecting wipes. Afterwards, numerable studies have demonstrated the efficacy of disinfecting

wipes based on the three-step protocol proposed by William et al. Siani et al. (2011) tested 9 commercially available wipes from different manufacturers. Their study revealed the importance of application time in the sporicidal activity of disinfecting wipe [186]. However, they did not investigate the role of wiping materials in conjunction with surface disinfectants. One innovation of their study is that they found spore binding to the wipe fibres, which gives more clues about the role of wiping materials in the disinfection process. Also, the authors introduced the strategy "one wipe, one application, one direction". Findings from Cadnum et al. gave a clear image of the efficient transfer of *C. difficile* spores from contaminated to clean surfaces using non-sporicidal wipes and the consistent reduction of *C. difficile* spores to undetectable levels at the inoculum site, with no transfer of spores to clean sites, using pre-moistened germicidal wipes [29]. However, the active ingredient of non-sporicidal wipes was not reported.

After several studies with the Williams' three-step protocol, it has been converted to the ASTM Standard E2967-15. The same year, Sattar et al. (2015) have published a paper regarding the efficacy of bioburden control of surfaces following disinfectant wipes use based on the new ASTM standard E2967-15. Five commercially available wipes have been tested with *Staphylococcus aureus* (ATCC 6538) and *Acinetobacter baumannii* (ATCC 19568) and their performance have been compared [51]. One advance of this research is the newly added drying process, which eliminates the detrimental influence of it on microbial viability. Again, the combination of wiping materials and active ingredients is randomly reported, therefore the study of the interaction issue remains vague. Hernandez et al. (2008) have studied the disinfection performance of chlorine dioxide imbibed wipes against *Mycobacterium avium* based on the European standard prEN 14563 carrier test [45]. However, their study was mainly focusing on mechanical action in the use of disinfecting wipes. There is more than one test method to assess the decontamination efficacy of disinfecting wipes. Gold et al. (2013), in their study have measured the cleanness, bacterial removal, and the force to remove the dried debris. Six commercially available disinfectant wipes were tested [187]. The innovation part of their research is that they also evaluate the force and time required by the disinfectant cleaning wipes to remove the debris from the surface. However, the measurement methods (OPA assay and ATP bioluminescence assay) they adopted seem not to be very accurate. It is a case study with difficulties to apply for general use. Nevertheless, this study gives hints for the selection of the disinfectant cleaning wipes. The case study in MRSA-positive hospitalized patients from Cheng et al. (2011) has evaluated the effectiveness of disinfection with wipes against methicillin-resistant *Staphylococcus aureus* (MRSA) [31]. Unfortunately,

their experiment design showed a critical drawback. The post-disinfection swab only contained sterile saline solution instead of a neutralizer to counteract the sporicidal action from the disinfectant agent after one prescribed contact time.

The impact of pathogen transfer from fomites to fingers, using surface disinfecting wipes, has been evaluated by Lopez et al. (2014) in their research. Their study tested three different surfaces with four types of microorganisms, *E. coli, S. aureus, B. thuringiensis*, and PV-1. Their study has found that some microorganisms may be more resistant to physical removal than others [53], which gives the idea that the adhesion of microorganisms on wipes may be different depending on the type of material used.

The impact of the interaction between wiping material and surface disinfectant on the decontamination efficacy of disinfecting wipes was finally taken into account in the work of Engelbrecht et al. (2013). They have tested both cotton and microfiber towels on their abilities to quats-binding using three different contact times. The study result indicated the reduction of quats concentration when exposed to cotton fibres, causing the disinfectant to fail the AOAC 961.02 Germicidal spray tests (GSTs) [46]. Unfortunately, the microbiology tests they performed did not test the disinfecting wipe in their field use, because the AOAC 961.02 GST does not consider the wipe in function of the microorganism removal during the wiping process. Thus, their study proved the deactivation of quats when exposing to cotton towels, but not the decontamination performance of quats disinfecting cotton towels. A list of the most important disinfecting wipes decontamination efficacy tests in literature is summarised in Table 6.

Table 6. Disinfecting wipes decontamination efficacy tests in literature.

Test organism	Textile substrate	Active ingredient	App. type	Surfaces	Contact time	Test method	Ref.
E. coli, P. aeruginosa, S. aureus, Streptococcus faecalis	(a) Heavy-duty paper wipe; (b) Non-woven rayon; (c) Non-woven fabric sheet	(a) 30% ethyl alcohol; (b) 10% ethyl alcohol and cetrimide; (c) Quaternary ammonium compounds	(a,b) PIDW (c) PSDW	Formica boards	Until dry	Swabbing techniques are superior to agar-impression methods	[41]
Adenovirus 8	(1) Pad, (2) Gauze, (3) Pad	(1) 70% isopropyl alcohol, (2) 3% hydrogen peroxide, (3) Iodophor	PSDW	Goldmann tonometer and pneumotonometer tips	5 s for wiping	Quantitatively assayed for residual virus	[64]
Meticillin-resistant (MRSA) or -susceptible (MSSA) *S. aureus*	n.a	Grapefruit extract	PIDW	Stainless steel discs	10 s rotation	Three-step protocol	[73]
Clostridium difficile	CAWP	Hypochlorite, QACs	PIDW	Steel discs	10 s rotation	Three-stage protocol	[186]
S. aureus (ATCC 6538) ***Acinetobacter baumannii (ATCC 19568)***	CAWP	H2O2, chloride and chloramine compounds; Sodium hypochlorite 1000 ppm, isopropanol; ethanol, quaternary ammonium compounds	PIDW	Discs (AISI Type 430; 1 cm in diameter and 0.7 mm thick) of magnetized and brushed stainless steel	10 s rotation	ASTM Standard E2967-15	[51]
Mycobacterium avium	Ready-to-use wipe	Chlorine dioxide concentration in the activated wipe was 200 ppm.	PIDW	Sterile frosted glass	30 s and 1 mins	prEN 14563	[45]

Coagulated blood test soil, *Streptococcus pneumoniae*	6 CAWP	Sodium hypochlorite, hydrogen peroxide, QACs, isopropanol	PIDW	Anesthesia machine surface	n.a	Residual protein debris by o-phthaldialdehyde analysis, bacterial survival by adenosine triphosphate measurement, measure of force required to remove the dried debris	[187]
MRSA-*positive hospitalized patients*	Disposable and non-disposable wipes (100% cotton)	1000 ppm hypochlorite	PIDW PSDW	Bed rails	5 mins	Five-steps method (more information can be find in the article)	[31]
E. coli, S. aureus, Bacillus thuringiensis spores, poliovirus 1	CAWP	Quaternary ammonium compounds (QACs)	PIDW	Ceramic tile, laminate, and granite	10 mins	Concentrations of transferred microorganisms on the fingers after the disinfectant wipe intervention	[53]
S. aureus (ATCC 6538), Salmonella enterica (ATCC 10708), P.aeruginosa (ATCC 15442)	Cotton and micro-fibre towels	Quaternary ammonium compounds (QACs)	PSDW	Glass slides	Less than 10 mins	AOAC International method 961.02 Germicidal spray tests (GSTs)	[46]
Campylobacter jejuni	n.a	n.a	PIDW	Ceramic tile, laminate and granite	n.a	Quantitative microbial risk assessment (QMRA)	[44]

Note: *E. coli: Escherichia coli; S. aureus: Staphylococcus aureus; P. aeruginosa: Pseudomonas aeruginosa;* CAWP: commercially available wipe product; PIDW: pre-impregnated disinfecting wipe (pre-wetted disinfecting wipe); PSDW: pre-soaked disinfecting wipe (bucket method); n.a. Not available.

2.8. Plasma treatment

Plasma treatment has been extensively studied and applied for surface modification of textile material. It is a dry (without wet polluting chemicals), environmentally- and worker- friendly method to achieve surface alteration without modifies the bulk properties of different materials. For textile industry, non-thermal plasma treatment is frequently used because most textile material cannot withstand the high temperature from the thermal plasma treatment [130]. Therein, atmospheric plasma and vacuum or low-pressure plasma treatments can be applied depending on the type of textile substrate and the desired assets. Various kinds of applied plasma gases were reported in the literature, such as oxygen (O_2), argon (Ar), carbon dioxide (CO_2), nitrogen (N_2), atmospheric air, etc. [188-190]. However, due to the limited application scale, relatively long treatment time, and expensive vacuum equipment requirement, low-pressure plasma is not competitive with the atmospheric plasma treatment from an economic point of view [191]. Additionally, atmospheric plasma can be integrated into textile production on-line, as an important advantage for its industrial application. The most commonly used atmospheric plasma treatment is the use of dielectric barrier discharge (DBD), corona discharge plasma treatment and atmospheric pressure jet (APPJ). In the last years, atmospheric cold plasma was widely employed because it does not need expensive vacuum equipment and allow continuous and uniform processing of fibres surfaces. Corona discharge has been used to enhance the adhesion of silicone-organic coating to polyamide fibre surfaces [130]. Corona plasma altered the surface roughness and surface free energy as well as created functional polar groups on the fibre surface [192]. Both surface activation and deposition of functional coatings through DBD atmospheric pressure plasma have been investigated in the previous studies to confer to textiles various properties, such as affinity for painting and dyeing, stain-resistance, antibacterial, no-shrinking and no-felting character [193]. DBD plasma is able to easily modify the surface of a wide range of natural and synthetic materials such as polyester, polyamide, wool and silk, improving the surface energy and surface oxidation, however, for cellulose material, usually only etching effect in terms of surface ablation and surface morphology change is expected [191, 194]. Moreover, it was recently reported that DBD plasma not only effectively cleans cotton fibres, increasing roughness and wettability of cotton sample but also increases polar surface functional groups and changes the surface charge [195]. Another research from Kusano et al has detected the decrease of C–C, C–H, and C–O (single bond) contents while an increase in C=O (carbonyl) and COO (carboxyl) contents in cellulose nanofiber surface [196].

All the possible changes resulting from plasma treatment could have an impact on the adsorption of QACs onto cellulose material. However, almost nothing is known about properties, performance, and disinfection efficiency of plasma-treated wipes. Therefore, DBD plasma treatment is considered as a potential solution to intervene in the interaction of QACs onto wipe materials and thereby improve antimicrobial efficacy [197].

2.9. Summary of the state of the art

Surface disinfectants integrated with textile materials as disinfectant-impregnated wipes are the most efficient and prevalent methods for the decontamination of high-touch environmental surfaces and non-critical medical devices in hospitals and other healthcare centres. There is evidence to support the significance of disinfecting wipes in preventing cross-contamination and spread of HCAIs. Despite this, less is known concerning the effectiveness of disinfecting wipes in the decontamination process. From the studies, several variables influence the disinfection efficacy of DIWs besides the external factors, these include:

- Disinfectant (type, concentration)
- Wipe (material, construction)
- Interaction between disinfectant and wipe
- Application method
- Wiping strategy including the applied pressure force, wiped surface area, the geometry of the mechanical action, number of passages, and remaining time on the surface.
- Storage time (function degradation)

Amongst, the interaction between disinfectant and textile substrate is the biggest encumbrance for its disinfection performance. Though, literature has revealed that an inappropriate material of the wipes could interact with the adsorbed active ingredient resulting in lower or even abolished disinfectant efficacy. At present, there is no clear understanding of the interaction phenomenon. More investigation is in need to obtain consistent and exhaustive knowledge about the interaction, in particular, examining and improving the following issues:

- Material compatibility (the combination of wipe and disinfectant)

- Liquor ratio (wipe mass/disinfection solution volume)
- Contact time (of disinfectant and wipes)
- Storage time

Besides, the standards to date remain some drawbacks in testing the effectiveness of DIWs. For example, difficulties exist in differentiating between mechanical removal of inoculum from a surface and chemical inactivation of the test microbe (High risk of cross-contamination when pathogens are just being removed by the wipe instead of being killed by the disinfectant greatly depending on the materials compatibility). More realistic disinfectant volume per unit surface area needs to be improved and applied. Divergent outcomes with different test standards can be suspected. A guideline for comparable results between various test standards is in demand. Nowadays, the most reliable method that can be used in hospitals seems to be the one using ready-to-use disinfecting wipes because of its lower disinfection failure risk. Due to the incomplete study of the decontamination efficacy of DIWs and the lack of testing standards validating the efficacy of DIWs in nosocomial practice, one can hardly advocate for their use in hospitals.

Chapter 3

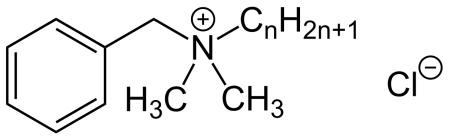
Materials and methods

3. MATERIALS AND METHODS

3.1. Materials

The quaternary ammonium salt used for the tests is alkyldimethylbenzylammonium chloride (ADBAC) and it was purchased from the company EMD Millipore Corporation, a subsidiary of Merck KGaA, Germany and reserved in a plastic bottle in solid status. The chemical formula is $C_6H_5CH_2N(CH_3)_2RCl$ (where R=C_8H_{17} to $C_{18}H_{37}$). Once the bottle was open, it was reserved in the desiccator containing silica gel desiccants to avoid its degradation from humidity. Two concentrations of QACs were used for different tests. The concentration of the solutions was calculated by measuring the UV absorbance of the solutions.

The concentration of ADBAC solution unspecified in the tests was prepared freshly with the concentration of 0.8 g L^{-1} (C_0) either by adding 0.2 g (±0.5%) of ADBAC to a 250 mL volumetric flask or by adding 0.8 g (±0.5%) of ADBAC to a 1000 mL volumetric flask filled up with distilled water. Other used concentrations will be singled out in the specific test. Figure 1 and Figure 2 exhibit the chemical structure and Fourier transform infrared spectroscopy (FTIR) fingerprint profile of ADBAC.



n = 8, 10, 12, 14, 16, 18

Figure 1. Chemical structure of ADBAC.

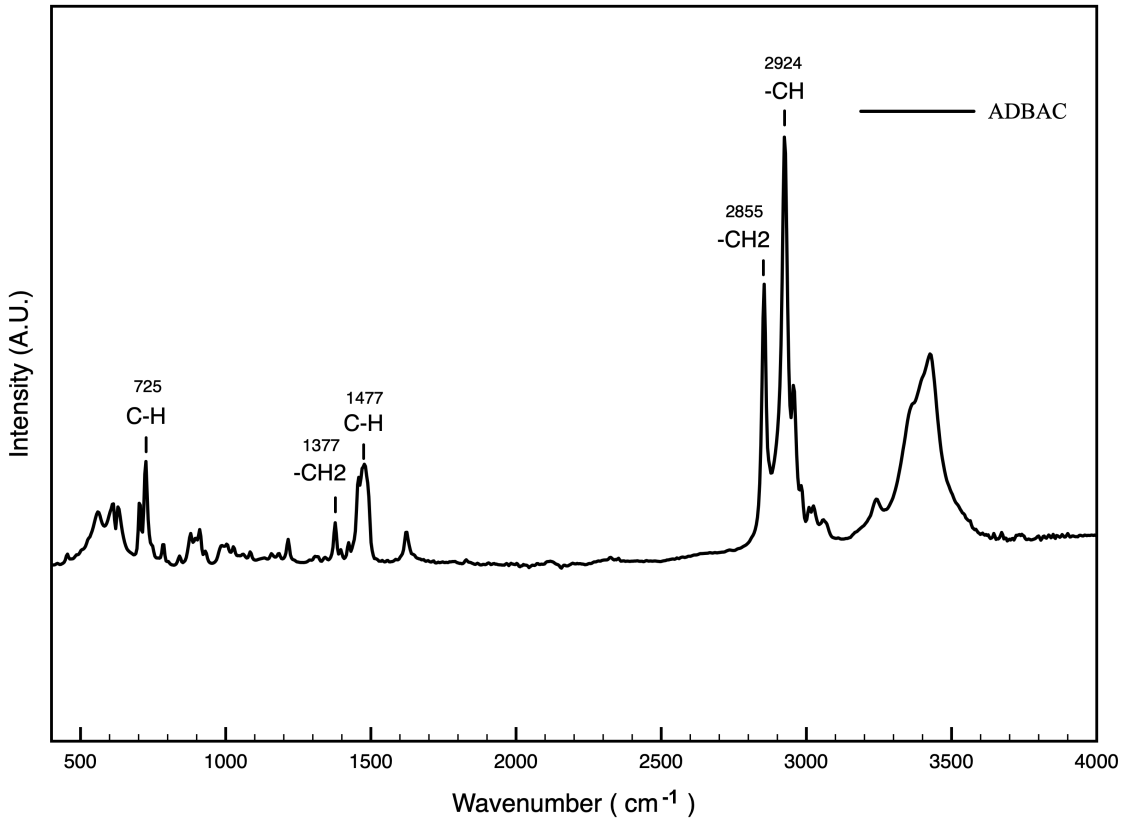


Figure 2. Diamond ATR-FTIR spectrum ADBAC in the full range of 400 to 4000 cm^{-1}.

The commercial wipe samples (Table 7) used in this project belong to the category of disposable and semi-disposable wiping cloths (W1 and W2 with areal density of below 75 g m^{-2}). W1 is TX409 Absorbond® (Texwipe Inc., Kernersville, North Carolina 27284, United States) and W2 is Wipe EcoCloth (Contec Inc., Spartanburg, South Carolina 29304, United States). White bleached cotton woven fabric with a warp density of 34 threads cm^{-1}, a weft density of 30 threads cm^{-1} was used.

To obtain the final wipes used for the project, a pre-selection of wipe samples was carried out. Material characterisation of all wipe sample candidates, such as areal density (Table S1), fabric thickness measurement (Table S2), air permeability (Table S3), Coefficient of friction (Table S4 and S5), vertical and horizontal wicking test measurement (Table S6 and S7), contact angle and surface energy (Table S8), and Differential Scanning Calorimetry (DSC) results (Figure S1-8), was presented in Annex I.

All other chemicals were purchased from Sigma-Aldrich and used without further purification steps.

3.2. Sample characterisation

Three wipes with different characteristics were selected for the tests (Table 7). Wipes were pre-washed with 0.05% non-ionic detergent Diadavin UN (Tanatex chemicals) in a standard washing process carried out by a long bath equipment, model IBELUS IL-720 (Labelus) integrated with an infrared heating system. The program started at 20 °C and rose up to 40 °C with a gradient of 3 °C min^{-1}, and the temperature remained for 30 minutes. The liquor ratio for the washing process is 1:100 (fabric mass in g: detergent solution in mL) with an agitation speed of 40 rotations per minute (rpm). Then, the samples were rinsed with distilled water by 1:100 liquor ratio three times. Afterwards, wipe samples were placed in an oven at 40 °C for 24h to dry for further use. Fabric thickness and areal density were determined at the standard atmosphere of 20 ± 2°C and 65 ± 2% relative humidity (RH). All wipe samples were conditioned for 48 hours before testing. The thickness of the fabrics was measured according to the standard ASTM D1777-96 (2015) with the digital thickness gauge M034 A at a pressure of 100 Pascal. Every sample was repeated 10 times and the mean and coefficient of variance (CV) were calculated (Table 7). A circular cutter with a surface area of 100 cm^2 was used to prepare the sample wipes for further areal density measurements. Every sample wipe was taken 5 times measurement. The data are reported as the average of the repetitions including their coefficients of variance in percentage (CV%) (Table 7).

Table 7. Information of material, structure, dimension, mean of fabric thickness and areal density and their coefficients of variance in percentage (CV%).

Sample	Components	Structure	Dimension (cm) of a 0.05 g sample	Fabric Thickness (mm) (CV%)	Areal Density (g m^{-2}) (CV%)
W1	100% polyester	Hydroentangled nonwoven	2.5 × 4.5	0.36 (5.93)	40.92 (1.59)
W2	55% cellulose 45% polyester	Hydroentangled nonwoven	2.5 × 2.5	0.54 (3.02)	69.58 (0.96)
W3	100% bleached cotton	1/1 plain weave	2.5 × 1.5	0.98 (5.09)	118.72 (0.41)

3.3. Spectrophotometric assessment of ADBAC concentration

UV-Vis spectrophotometry (Shimadzu, UV-1800) was used to measure the concentration of ADBAC with a standard quartz cuvette. Initially, the λ_{max} was identified through a full wavelength scans from 200 - 800 nm. Three peaks at 268.5, 262.5, and 256.5 nm were detected. The wavelength at 262.5 nm was eventually employed because of its highest response and a corresponding calibration curve of concentration [C] versus Absorbency (Abs) was developed using 5 different concentrations of ADBAC in distilled water (0, 0.2, 0.4, 0.5, 0.8, 1 g L^{-1}) and shown in equation (1):

$$[C_{ADBAC}] = 0.806\ Abs - 0.007 \qquad (R^2 = 0.9999) \qquad (1)$$

The generated equation (1) was used to calculate the concentration of ADBAC solution in the subsequent tests with the obtained Abs values from the UV-Vis spectrophotometry.

3.4. DBD plasma treatment of wipe samples

The dielectric barrier discharge (DBD) plasma treatment was conducted in a semi-industrial prototype (Softal Electronics GmbH/University of Minho, Braga, Portugal) working at room temperature and atmospheric pressure, using a system of metal electrode coated with ceramic and counter electrodes coated with silicon, with 50 cm effective width, gap distance fixed at 3 mm, and producing the discharge at high voltage 10 kV and low frequency 40 kHz. The machine was operated with the optimized parameters: 1 kW power, 4 m min^{-1} velocity, 5 passages corresponding to a dosage of 2.5 kW min m^{-2} adopted from the previous study [132]. Plasmatic dosage was defined by equation 2:

$$\text{Dosage} = \frac{n \cdot p}{v \cdot l} \qquad (2)$$

where, n = number of passages, p = power (kW), v = velocity (m min^{-1}), and l = width of treatment (0.5 m). The wipe was passed through a laminar plasma between a cylindrical silicone rotating drum and

ceramic electrodes for one side then treated on the other side. A schematic diagram using a photo of the used equipment was provided in Figure S9 in the Annex II.

3.5. Absorption and adsorption tests

Wiping materials, immersion time and liquor ratio (fabric mass g: bulk solution ml) variables were studied. Plasma-treated and untreated wipe samples were studied at four different immersion times (10, 30, 60, and 90 min) and eleven sets of liquor ratios (fabric mass g: bulk solution ml). The volume of the ADBAC bulk solution was fixed to 10 ml. Thus, the corresponding fabric mass (M_w) to certain liquor ratio is listed in Table 8.

Table 8. Information of fabric mass to corresponding liquor ratio, data represents mean values with CV±5%.

Liquor ratio	1:10	1:15	1:20	1:25	1:30	1:40	1:60	1:80	1:100	1:120	1:200
Fabric mass (g)	1.00	0.67	0.50	0.40	0.33	0.25	0.17	0.13	0.10	0.83	0.05

10 mL of freshly prepared stock solution (C_0) was pipetted in every high-density polypropylene conical tubes. This volume of the stock solution was converted in grams (W_0) assuming a density of 1 g L^{-1} for the ADBAC solution. Then, different wipe sample masses (M_w) were immersed to achieve the corresponding liquor ratios. The wipes were immersed for a specific time and then removed with tweezers. The concentration of the remained solution (C_t) after removing the wipe was then measured. The weights of the tube with the liquid were recorded before the wipe immersion (S_0), during the wipe immersion (S_w) and after removing the wipe (S_t). The calculated ADBAC mass was later converted to volume (mL) assuming a density of 1 g L^{-1} for the ADBAC solution. Graphical representation of the immersion process can be found in Figure S10 in Annex II. For every liquor ratio and immersion time, 3 independent replicates and 1 water control were performed.

3.6. Calculation of the concentration of ADBAC in the wipe (C_Q) and of the weight ratio between the amount of ADBAC on the wipe and the wipe mass (R_w)

The step by step calculation of C_Q (equation 3) and R_w (equation 4) is presented in this section and its graphical representation is presented in Figure S11 in Annex II. The elements involved in the calculation are defined, summarized and listed in Table 9, Table 10, and Table 11.

Table 9. Elements obtained by direct measurement.

Symbol (Unit)	**Definition**
S_0 (g)	The initial total weight (Tube + ADBAC solution)
S_W (g)	Initial total weight with immersed wipe (Tube + ADBAC solution + Wipe)
S_t (g)	Total weight after removing the wipe (Tube + ADBAC solution - Wipe)
V_0 (mL)	Volume of the initial ADBAC bulk solution (10 mL)
W_0 (g)	Weight of the initial ADBAC bulk solution converted from **V_0** assuming a density of 1 g L^{-1} for the ADBAC solution (10 g)
Abs_0	Absorbance of the ADBAC stock solution
Abs_t	Absorbance of the ADBAC solution after removing the wipe

Table 10. Elements obtained by primary calculation.

Symbol (Unit)	**Definition**	**Calculation**
W_W (g)	Weight of the solution absorbed by the wipe sample	$S_0 - S_t$
V_W (mL)	Volume of the solution absorbed by the wipe sample converted from **W_w** assuming a density of 1 g L^{-1} for the ADBAC solution	$S_0 - S_t$
M_W (g)	Initial weight of the wipe sample	$S_W - S_0$

C_0 (g L^{-1})	Initial concentration of the ADBAC stock solution	0.806 * Abs_0 - 0.007
C_t (g L^{-1})	Concentration of the ADBAC solution after removing the wipe	0.806 * Abs_t - 0.007

Table 11. Elements obtained by secondary calculation.

Symbol (Unit)	**Definition**	**Calculation**
W_t (g)	Weight of the ADBAC solution after removing the wipe	$W_0 – W_w = W_0 - (S_0 - S_t)$
V_t (mL)	Volume of the ADBAC solution after removing the wipe converted from **W_t** assuming a density of 1 g L^{-1} for the ADBAC solution	$W_0 – W_w = W_0 - (S_0 - S_t)$
Q_0 (g)	ADBAC amount in stock solution	$C_0 * V_0 = (0.806 * Abs_0 - 0.007) * V_0$
Q_t (g)	ADBAC amount remained in solution after removing the wipe	$C_t * V_t = C_t * (V_0 – V_w) = (0.806 * Abs_0 - 0.007) * (V_0 – V_w)$
Q_W (g)	ADBAC amount absorbed in the wipe sample	$Q_0 - Q_t$

Based on the description above, the concentration of ADBAC active ingredients adsorbed in the wipe samples (C_Q) and active ingredient weight ratio absorbed by the wipe (R_w) are calculated as follow:

$$\mathbf{C_Q = Q_W/V_W}$$

$Q_w = (Q_0 - Q_t)$; $Q_0 = C_0 * V_0$; $Q_t = C_t * (V_0 - V_w)$;

$C_Q = (C_0 - C_t) * V_0/V_w + C_t$

$$\boldsymbol{C_Q} = C_0 \times \frac{V_0}{V_W} - C_t \times (\frac{V_0}{V_W} - 1) \quad (3)$$

$$\mathbf{R_W = Q_W/M_W}$$

$Q_w = (Q_0 - Q_t)$; $Q_0 = C_0 * V_0$; $Q_t = C_t * (V_0 - V_w)$;

$R_w = [(C_0 - C_t) * V_0 + C_t * V_w]]/M_w$

$$\boldsymbol{R_W} = C_0 \times \frac{V_0}{M_W} - C_t \times (\frac{V_0}{M_W} - \frac{V_W}{M_W}) \quad (4)$$

3.7. Contact angle and surface free energy measurement

An OCA 15 apparatus (Dataphysics Instruments GmbH, Filderstadt, Germany) with OCA20 software was used to characterise the stationary and dynamic contact angles (SCA and DCA) and surface free energy of sample wipes. Each measurement was repeated five times and the average and standard deviation (SD) were calculated. Three liquids with known surface free energy and surface free energy components were used for the calculation of surface free energy, namely: distilled water (γ 72.8, γ^D 29.1, γ^P 43.7) with a dosing volume of 5 µl and dosing rate of 5 µl s^{-1}, polyethylene glycol 200 (PEG) (γ 43.5, γ^D 29.9, γ^P 13.6) with a dosing volume of 5 µl and dosing rate of 12 µl s^{-1}, and glycerol (γ 63.4, γ^D 37.4, γ^P 26.0) with a dosing volume of 5 µl and dosing rate of 12 µl s^{-1} [198]. The surface free energy (γ) was defined by polar and dispersive components. Principally, permanent and induced dipoles and hydrogen bonding result in three different intermolecular forces that composed the polar component, whereas the dispersion (non-polar) component of γ results from instantaneous dipole moments. The calculation of polar and dispersive components of the surface free energy (γ^D and γ^P, respectively) is based on the Wu method (harmonic mean) by equation 5:

$$\gamma_{sl} = \gamma_s + \gamma_l - 4\left[\frac{\gamma_s^D \gamma_l^D}{\gamma_s^D + \gamma_l^D} + \frac{\gamma_s^P \gamma_l^P}{\gamma_s^P + \gamma_l^P}\right] \quad (5)$$

For polar solids or liquids, the total γ, defined by equation 6, is a sum of the always-existing London dispersion forces (γ^D) with intermolecular interactions that depend on the chemical nature of the material, compiled as polar forces (γ^P):

$$\gamma = \gamma^D + \gamma^P \quad (6)$$

The work of adhesion (W_{Adh}), represents the energy of interaction between the liquid and the solid phases per unit area, as defined by equation 7.

$$W_{Adh} = \gamma_l (1 + \cos\theta) \quad (7)$$

where θ is the water contact angle calculated by goniometer and γ_l means the 'liquid' surface free energy, which is calculated by equation 6. Each combination of wipe sample and liquid in the measurement of SCA and DCA were carried out 10 times and 3 times, respectively. The data is presented as mean ± standard deviation (SD).

3.8. Laser scanning microscope (LSM)

The untreated and plasma-treated wipe samples were analysed with an LSM from Keyence microscope (Osaka, Japan), model VK-X160 equipped with a red semiconductor laser supplied at the wavelength of 658 nm. The parameters of arithmetical mean height (Sa), maximum height (Sz), arithmetic mean peak curvature (Spc), and developed interfacial area ratio (Sdr) were measured to evaluate the surface roughness. Scanning was stitched 80 times composing an area of $10mm^2$.

3.9. X-Ray photoelectron spectroscopy (XPS)

The XPS analyses were carried out on the PHI-TFA XPS spectrometer produced by Physical Electronics Inc. (Chanhassen, EUA) to determine the surface functional groups. Samples were mounted on the metallic sample holder and introduced in ultra-high vacuum spectrometer. The vacuum during the XPS analyses was in the range of 10^{-9} mbar. Sample surfaces were excited by X-ray radiation over a 0.4 mm spot area (about 3-5 nm in analysis depth) with a monochromatic Al source at photon energy of 1486.6 eV. The survey wide-energy spectra were taken over an energy range of 0-1400 eV with pass energy of analyser of 187 eV to identify and quantify present elements on the surface. All the measures were taken with an angle tilt of 45°. The high-energy resolution spectra were acquired with energy analyser operating at pass energy of 29 eV with a resolution of about 0.6 eV. During data processing, the spectra were aligned by setting the C1s peak at 284.8 eV, characteristic for C-C/C-H bonds. The accuracy of binding energies was about ± 0.3 eV. Two places on every sample were analysed and average composition was calculated. XPS spectra were analysed for elemental composition by a Multipak

software, version 8.0 from Physical Electronics Inc. (Chanhassen, EUA). Deconvolution into sub-peaks was performed by least-squares peak analysis software, XPSPEAK version 4.1, using the Gaussian/Lorentzian sum function and Shirley-type background subtraction. The peaks were constrained to have equal FWHM (Full Wave at Half Maximum) to the main peak. No tailing function was considered in the peak fitting procedure. The components of the various spectra were mainly modelled as symmetrical Gaussian peaks unless a certain degree of Lorentzian shape was necessary for the best fit.

3.10. Storage of wipe samples in ADBAC solution

The wipe samples were immersed in the prepared ADBAC solution (C_0 = 0.8 g L^{-1}) in the liquor ratio of 1:20 (fabric mass g: bulk solution ml). To fulfil the sample requirement from UTM measurement, two pieces of wipe sample with 0.5 g mass were immersed in 20 mL ADBAC solution for both directions (cross and machine directions for W1 and W2 or warp and weft directions for W3). Wipe samples and ADBAC solution were maintained in a 50 mL conical tubes and stored in a shaded, cool, dry, and well-ventilated cabinet for 30min, 1 day, 3 days, 7 days and 30 days. When the due time elapsed, wipe samples were taken out by tweezers and dry in the oven at 40 °C for 24 hours for further tests i.e. FTIR, DMA, breaking force and elongation measurement. The remained ADBAC solution was analysed by UV-Vis spectrophotometry. For ASTM E 2149-13a test, wipes were cut in samples of 0.05 gram (Table 7). Six pieces of the same wipe samples were immersed in 6mL ADBAC solution in a 15 mL volume high-density polypropylene conical tubes with an adjustable pipette (1 to 10 mL). Every wipe sample set was coupled with water control under the same experimental conditions.

3.11. Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra of the wipe samples before and after treatment were recorded using an IR-Affinity 1 FTIR spectrophotometer (Shimadzu, Kyoto, Japan) with an attenuated total reflectance accessory (ATR) to determine the surface chemical changes. Spectra were collected at room temperature in the spectral

range of 4000-700 cm^{-1} at the resolution of 4 cm^{-1} and summations over 45 scans. All the samples were dried in an oven at 40°C for 24h prior testing.

3.12. Breaking force and elongation at break measurement

Fabric strain versus applied force and time was measured (at 20 °C and 65% RH) with a Universal Testing Machine (Model 4500, Instron Corporation, Norwood, Massachusetts, EUA) using a 250 N load cell at the crosshead speed of 1 mm min^{-1}. Samples of 2 x 10 cm were tested in warp and weft (or cross and machine) directions at the maximum load of 250 N using the standard test method for breaking force and elongation of textile fabrics (Strip Method, ASTM D 5035:11 2019) [199]. Wipe samples at all aged time were tested with three replicates of each sample.

3.13. Dynamic Mechanical Analysis (DMA)

DMA was performed using a DMA 7100 from Hitachi® (Tokyo, Japan) in programmed tension mode. The temperature dependence of the tan, storage and loss moduli were measured in the temperature range 30 to 200 °C, with a heating rate of 3 °C min^{-1}. The geometry of the testing sample was 20 mm in length, 10 mm in width and the thickness in mm of each wipe as reported in Table 7. Specimens were prepared in duplicate to conduct thermo-mechanical analysis. These analyses were carried out under nitrogen purge of 200 ml min^{-1}. 7 days' ADBAC/water immersed untreated and plasma-treated wipe samples were investigated by DMA. The wipe samples were dried in an oven at 40 °C for 24 h prior testing.

3.14. Microbiology test ASTM E 2149-13a

The ASTM E 2149-13a Standard Test Method for Determining the Antimicrobial Activity of Immobilized Antimicrobial Agents Under Dynamic Contact Conditions was modified to assess the interaction between textile substrate and active ingredients [200]. The most important modification of the standard

was the enhancing of the bacteria inoculum concentration in order to allow more evident distinction in differentiating the antimicrobial performance among the three wipe samples. Therefore, the bacteria inoculum concentration was rose up to 1.5 - 3.0 × 10^9 CFU mL^{-1}. However, other modifications with the intent of saving material due to the high amount of used samples and bacterial suspensions were made in terms of wipe sample size and volume of bacteria inoculum. The wipe sample size was defined at 0.05 g and the volume of the bacteria inoculum was reduced to 5 mL. Due to the different areal densities of wipe sample materials, the approximate dimension of wipe samples is reported in Table 7.

The working bacteria are *Staphylococcus aureus* (*S. aureus*), ATCC 6538 and *Escherichia coli* (*E. coli*), ATCC 25923 representing Gram-positive and Gram-negative bacteria, respectively. The stock culture was prepared from the freeze-dried ampule into several vials and stored at –80 °C. A subculture (G1) was prepared from the frozen stock culture on Tryptone Soya Agar (TSA) plates and incubated at 37 °C for 24 h. The working culture for the test is freshly prepared from G1 in the sterile Tryptic Soy Broth (TSB) for 18 h at 35 ± 2 °C prior to performing the test. The testing samples (different treatments based on different test purposes, detailed found below) with 5 mL bacterial suspension were maintained in a 15 mL sterilized conical tubes for 1 hour with orbital shaking at 120 rpm at 37 °C. Afterwards, liquids were collected and diluted from each tube for plating. Plates were incubated at 35 ± 2 °C for 18 - 20 hours for colony forming unit (CFU) counting. The plate counting, log reduction calculation and other detailed test procedure without specification were performed according to the standards. Graphical illustration of the shaking flask procedure can be found in Figure S12 in Annex II.

ASTM E 2149-13a Standard was performed for two tests with different research aims, applying two sample-settings accordingly:

i. ADBAC adsorption assessment by microbiology approach

Testing samples were prepared by pipetting 40 µL of 6300 µg mL^{-1} ADBAC stock solution onto wipe samples. 40 µl was chosen because all wipes (0.05 g) were able to absorb all the liquid without any remaining excess (confirmed from a preliminary test). In the bactericidal testing study of ADBAC, Ioannou, et al. adopted 35, 45, and 55 µg mL^{-1} as the final concentrations of ADBAC in the reaction vessel [201]. In this study, 50 µg mL^{-1} was designated for the final concentration in the bacteria suspension. The testing samples were prepared in three repetitions, 1 min before inserted in the bacteria suspension (5 mL). Every testing sample was complemented with a water control (40 µL

sterilized distilled water instead of ADBAC). Log reduction was calculated by subtracting the CFU Log data of distilled water from the CFU Log date of ADBAC.

ii. Evaluation of antimicrobial efficacy over storage time

In the evaluation of antimicrobial efficacy over storage time test, the testing samples were taken directly from previously immersed wipes in ADBAC solution (Materials and methods 3.10) over different storage time (30min, 3, 7, 15 and 30 days). The testing samples were also prepared in three replicates and coupled with water control.

3.15. Antimicrobial efficacy test of the eluate

The Eluate wrung out from the immersed wipe samples were tested with standard EN 13727:2012+A2:2015 Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of bactericidal activity in the medical area - Test method and requirements (phase 2, step 1) [202].

Three types of untreated wipe samples were cut in the weight of 3.5g and immersed in 20 mL ADBAC solution with concertation of 0.8 g L^{-1} (for *S. aureus*) or 1.6 g L^{-1} (for *P. aeruginosa*) in a 50 mL volume high-density polypropylene conical tubes with adjustable pipette (1 ml to 10 ml) for 20 hours overnight under room temperature. 20mL distilled water instead of the ADBAC solution was used as the water control in the test. Afterwards, the eluates were wrung out from the immersed wipe samples with a manual mini-wringer (Idserda products V.O.F. Wapserveen, the Netherlands). Meanwhile, the remaining liquid in the bulk was collected together with the eluates for their concentration measurement by UV-Vis spectrophotometry. Three-time repetitions were done. The concentration and concentration reduction of both eluates (E) and remaining bulk (B) liquid were recorded.

The collected liquid from the eluates was tested with standard EN 13727:2012+A2:2015. EN 13727 is a test standard including four types of test conditions, including i) hygienic handrub and handwash, ii) surgical handrub and handwash, iii) instrument disinfection and iv) surface disinfection. For this study, the test condition for surface disinfection is adopted. Thereby, *Staphylococcus aureus* (ATCC 6538) and *Pseudomonas aeruginosa* (ATCC 15442) were chosen correspondingly for the experiment. The interfering substance to be tested was 0.30 g L^{-1} bovine albumin representing the cleaning condition.

After a preliminary test, the number of *P. aeruginosa* cell in the testing suspension was designated to be between 1.5×10^{6} CFU mL^{-1} to 5.0×10^{6} CFU mL^{-1} due to the weak bactericidal ability of ADBAC against Gram-negative bacteria.

The procedure was modified as following: 8 mL testing bacteria suspension was pipetted into a glass tube following 1mL interfering substance (0.30 g L^{-1} bovine albumin) well mixed with contact time 2 min ± 10s. At the end of the time, 1 mL of the ADBAC solution was added into the mixture. Mixed well and leave for contact time 30 mins. At the end of 30 mins contact time, 1 mL sample of the test mixture was transferred into a tube containing 9 mL of neutralizer (Validated neutralizer recipe: polysorbate 80, 30 g L^{-1} + saponin, 30 g L^{-1} + lecithin, 3 g L^{-1}) well mixed for 5 min ± 10s. At the end of the neutralization, transfer 1 mL of the sample for further dilution or plating with pour plate technique in duplicate. The test was performed with three repetitions. Other procedure and testing conditions without specification followed the standard EN 13727. The Log reduction of the testing cell in the suspension was calculated in average and together with their standard deviation (SD) were reported.

3.16. Antimicrobial efficacy evaluation in practice with standard EN16615

Standard EN16615-2015 was selected to determine the antimicrobial efficacy of the selected wipe samples in practise [203]. As introduced in the state of the art, EN16615-2015 as a phase 2 step 2 test, could maximally simulate the use of pre-impregnated disinfecting wipe in practice among the other standards. Some modifications were made to achieve a valuable result for analysis. The concentration of QACs used in the test is 0.8 g L^{-1}. A preliminary test was performed and found 0.8 g L^{-1} can obtain distinguishable outcomes between three wipe types. The testing bacterium is *Staphylococcus aureus*, ATCC 6538. The concentration of the testing suspension is between 2.0×10^{8} CFU mL^{-1} to 8.0×10^{8} CFU mL^{-1}. Clean condition with 0.30 g L^{-1} bovine albumin was applied.

Due to the different water absorbency properties from the testing samples, to reach the same amount of disinfectant solution on the testing surface, different amounts of ADBAC solution were applied on each wipe samples. Using the standard wipe selected in EN16615 as a reference, the following Wipe/ADBAC (g/mL) sets were applied: W1/ADBAC - 3.0/16.0, W2/ADBAC - 3.0/16.0, W3/ADBAC - 3.0/5.0. The rest procedures without other specification followed the standard EN 16615-2015.

3.17. Statistical analysis

Experiments were performed in three replicates otherwise specified and the mean value, standard deviation (SD) of each data were reported. Two-way ANOVA analysis was applied to

i. Compare the mean differences between liquor ratio (column) and immersion time (row) as the two factors and C_R as the response variable;
ii. Compare the main population difference between the material type and bacteria type as two factors and bacteria Log reduction as the response variable;
iii. Analyse the effect from the storage time (factor) on the antimicrobial efficacy (response variable).

The level of significance (α) was set as 0.05. The null hypothesises are:

1) There is no significant effect of liquor ratio on the concentration reduction in the adsorption of ADBAC onto textile substrate;

2) There is no significant effect of immersion time on the concentration reduction in the adsorption of ADBAC onto textile substrate;

3) There is no significant difference in the bacteria Log reduction between different material types;

4) There is no significant difference in the bacteria Log reduction between different bacteria type (Gram-positive and negative);

5) There is no significant effect of storage time on the antimicrobial efficacy of disinfecting wipes.

When the p-value is smaller than α ($p<0.05$), the null hypothesis can be rejected.

Chapter 4

Results and Discussion

4. RESULTS AND DISCUSSION

It may be necessary to point out, the final wipe samples used for the experiment are a selection from the physical characterization result including areal density (g m^{-2}) (Table S1), fabric thickness measurement (mm) (Table S2), air permeability (L m^{-2} s^{-1}) (Table S3), Coefficient of friction (Table S4 and S5), vertical and horizontal wicking test measurement (Table S6 and S7), contact angle and surface energy (Table S8), and DSC results (Figure S1-8) of all wipe sample candidates in stock. All the detailed data and discussion can be found in Annex I Pre-selection of wipe samples. Eventually, three types of wipes (Table 7) have been selected for the investigation. This chapter presented the principal investigation result and discussion in 4 sections following the study of absorption and adsorption, chemical interaction analysis, ageing performance, and antimicrobial efficacy tests.

4.1. Absorption and adsorption

Textile-based materials in contact with an aqueous QACs solution are subjected to 2 main process: i) the absorption of the solution onto the textile material due to its absorbency characteristic, one of the most important physical properties of textile materials, and ii) the adsorption of active ingredients from the solution onto textile material, which can result from the adhesion of the molecule or chemical interaction [204]. Adsorption is intended as the accumulation of a substance (active ingredient) at the interface between a solid surface (textile) and the bathing solution (disinfectant). Both the absorption and adsorption of ADBAC onto the wiping materials were studied considering several parameters such as wiping material, immersion time, and liquor ratio. Wipe samples with different mass weights were immersed in the ADBAC solution for a certain time. Three variables were considered as the most important indicators for the disinfection efficacy: i) The absorption ability of the wipes, contributes to the liquid availability to the target surface; ii) The concentration reduction of ADBAC in the bulk solution ($\mathbf{C_R}$), due to the interaction between the wipe and disinfectant while contact; (iii) The concentration of ADBAC eventually absorbed in the wipe ($\mathbf{C_Q}$), which reflects the final concentration of disinfectant that can be applied on the target surface, assuming that all the active ingredient absorbed by the wipe will be released on the target surface; iv) The weight ratio ($\mathbf{R_W}$) that reflect the amount of active ingredient remaining on the wipe calculated as the amount of ADBAC absorbed in the wipe divided by the weight

of the wipe. $\mathbf{R_w}$ allows estimating how long the disinfection ability could be sustained during the wiping process.

4.1.1 Absorption ability of ADBAC on wiping materials

The absorption can have a significant impact on the disinfectant performance due to the amount of disinfectant solution that can be delivered to the target surface. The final effect will be more pronounced if most of the liquid absorbed is available on the wipe surface instead of the bulk. Fitting of the absorption result shows that the liquid absorbed by the wipe ($\mathbf{V_w}$) is in a linear relationship with the fabric mass ($\mathbf{M_w}$) independently of the used liquor ratio (1:200 up to 1:10) because the amount of liquid to be absorbed by the wipes in this experiment is always in excess (Figure 3). The slope of the linear fitting represents the water absorbency ability of the material. The higher the slope is, the better is the water absorbency property of the material. On one hand, control nonwoven wipes W1R and W2R displayed very similar values suggesting that pure polyester (W1R) and polyester/cellulose blend (W2R) has comparable volumetric absorption in this structural configuration (Figure 3-a). On the other hand, the woven fabric of pure cellulose (W3R) shows the lowest value mainly due to the higher degree of geometrical complexity. Differently to the homogeneous and structurally anisotropic nonwoven textiles, the water absorption of a woven fabric is mostly driven by the fabric thickness, solid volume fraction, and air permeability [205]. After DBD plasma treatment (Figure 3-b) W1P shows a slight increase in water absorbency clearly due to the plasma treatment that significantly enhances the polar component of polyester material. On the contrary, W3P displays a small decrease because of the surface etching effect of plasma treatment on the top layer of the cotton fabric [206]. W2P shows the most significant decrease suggesting that the cellulose micro-fibrillated moiety and the nonwoven geometry have a considerable impact on the liquid absorbency even if the polyester structure hydrophilicity was improved by the plasma treatment [191].

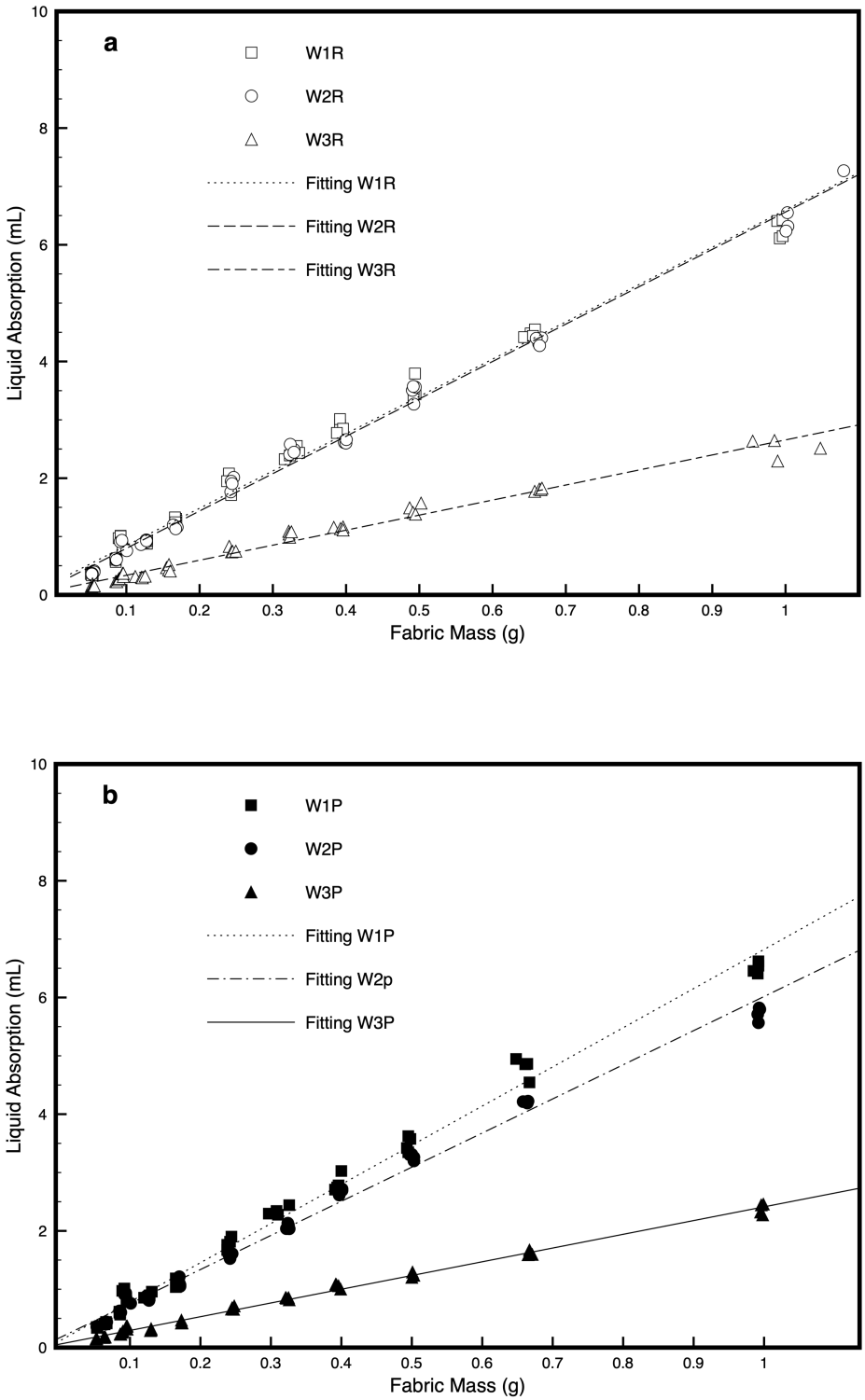


Figure 3. Correlation between fabric mass (X) and liquid absorption: a) for control wipe samples and b) for plasma-treated wipe samples (R^2=0.99). W1R=6.39X+0.21; W2R=6.41X+0.16; W3R=2.58X+0.08; W1P=6.71X+0.11; W2P=5.85X+0.17; W3P=2.35X+0.06.

4.1.2 Concentration reduction of ADBAC in the bulk solution (C_R)

The concentration reduction in bulk solution (**C_R**) is calculated by equation 8:

$$\boldsymbol{C_R} = 100 \times \frac{C_0 - C_t}{C_0} \quad (8)$$

The higher is the concentration reduction (**C_R**) value, the stronger is the adsorption of active ingredients in the solution. **C_R** is clearly dependent on the set of liquor ratio and immersion time. Detailed dataset of **C_R** on control and plasma-treated wipe samples regarding all the liquor ratios and immersion times can be found in Table S9 in the supporting information. On one hand, liquor ratio, as shown in ANOVA analysis (Table S10 in the supporting information), has a significant impact on the concentration reduction of ADBAC for all wipe samples in both untreated and plasma-treated samples. Moreover, in W2 and W3 wipes the **C_R** decreases with the reducing of liquor ratio following a S-type adsorption isotherm (in W1 the **C_R** is too small to see a trend). On the other hand, regarding immersion time, it is evident from the data that the adsorption of ADBAC took place in the first 10 mins after the wipe contact in both untreated and plasma-treated samples (Figure 4, Figure 5 and Figure 6). Excluding W1, which does not show significant adsorption of ADBAC, both W2 and W3 wipes presented an increase of **C_R** in function of the immersion time (see ANOVA analysis in Table S10 in the supporting information). For both untreated and plasma-treated samples from 10 mins to 30 mins, the **C_R** values showed a small increase in W2 and a significant increase in W3 wipe (Figure 5 and Figure 6) especially for the highest liquor ratios (e.g. 1/10 and 1/15) diminishing to a plateau at lower liquor ratios (e.g. 1/100, 1/120, and 1/200). Then, the adsorption slowed down showing no significant difference between immersion time at 60 mins and 90 mins.

Also the materials and plasma treatment have a significant effect on the **C_R** variability. W1 displayed a very low **C_R** value while after plasma treatment a slight increase of **C_R** was noticed meaning that a higher amount of ADBAC is present in the wipes. WR3 showed the highest **C_R** value, almost double than WR2, under the same condition (immersion time and liquor ratio) due to the different cellulose content (Figure 5 and Figure 6). This is following the literature showing significant binding of quaternary ammonium compounds by cellulosic material [46, 207]. Additionally, it was found that the binding degree is proportional to the content of cellulose in the fabric [55]. After plasma treatment, W3P displayed a significant reduction of the **C_R** compared with the W3R, while W2P showed an opposite behaviour (Figure 5 and Figure 6).

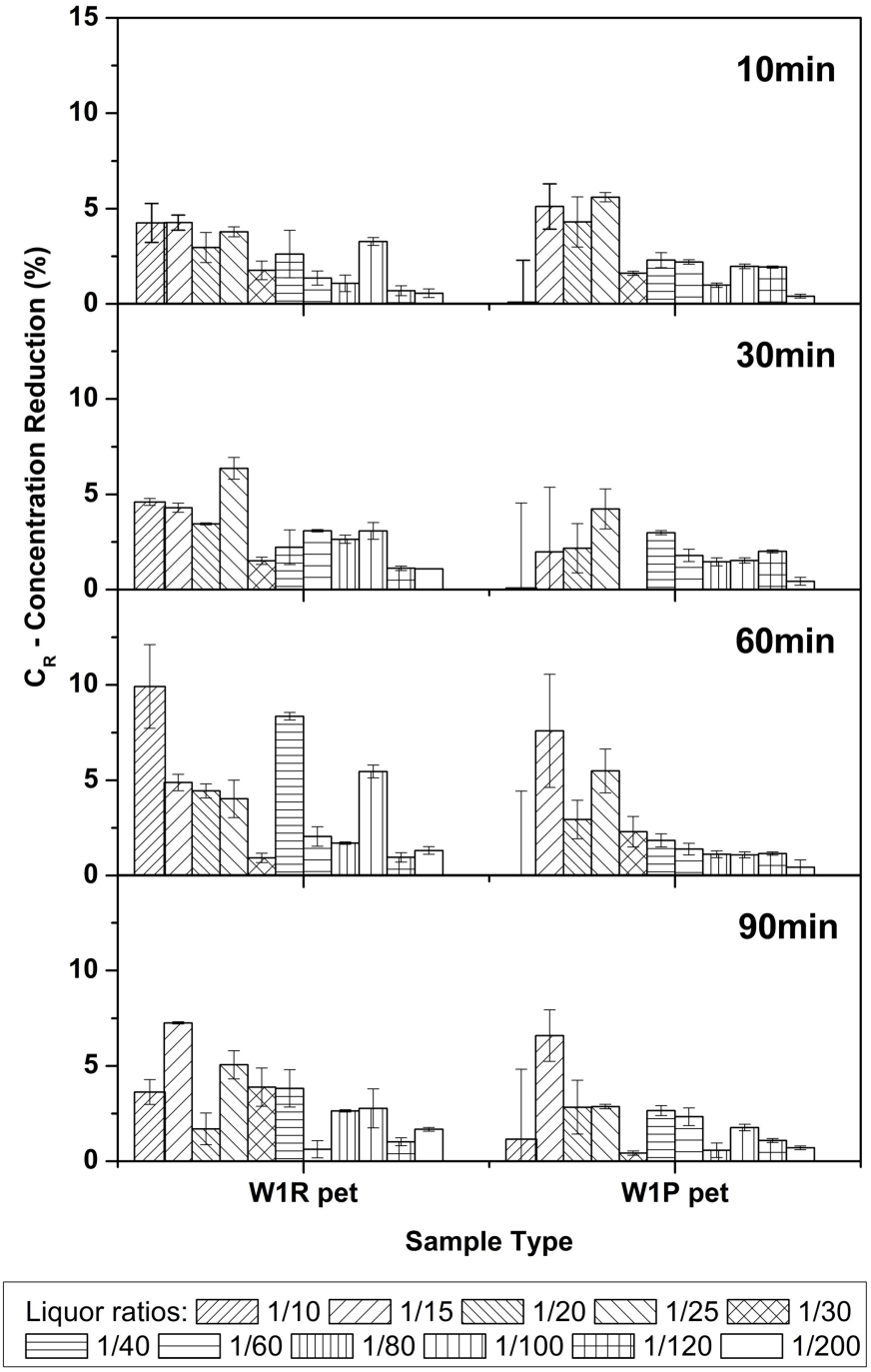


Figure 4. Concentration Reduction (C_R) ± SD of untreated (R) and plasma-treated (P) W1 samples changing with immersion time and liquor ratio.

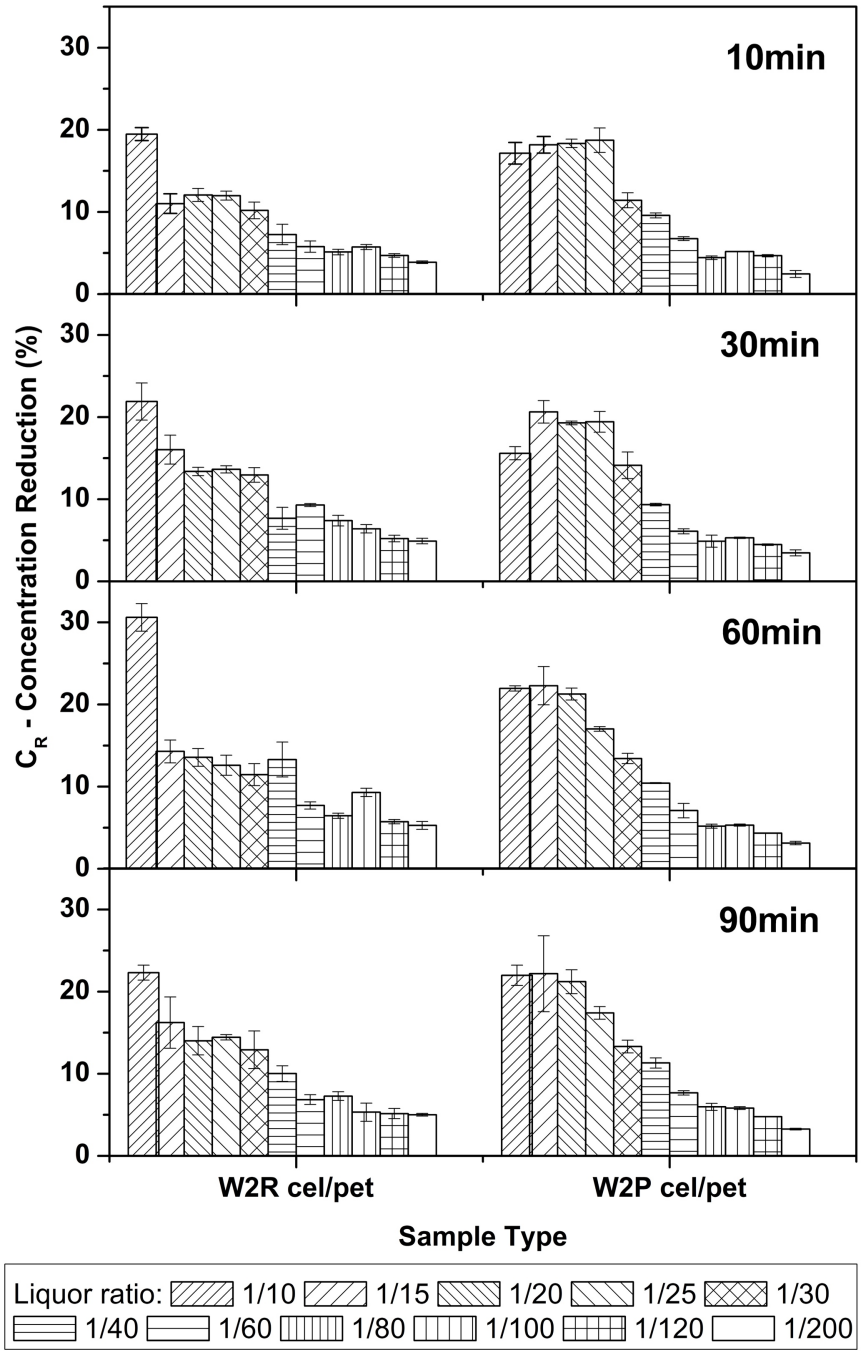


Figure 5. Concentration Reduction (C_R) ± SD of untreated (R) and plasma-treated (P) W2 samples changing with immersion time and liquor ratio.

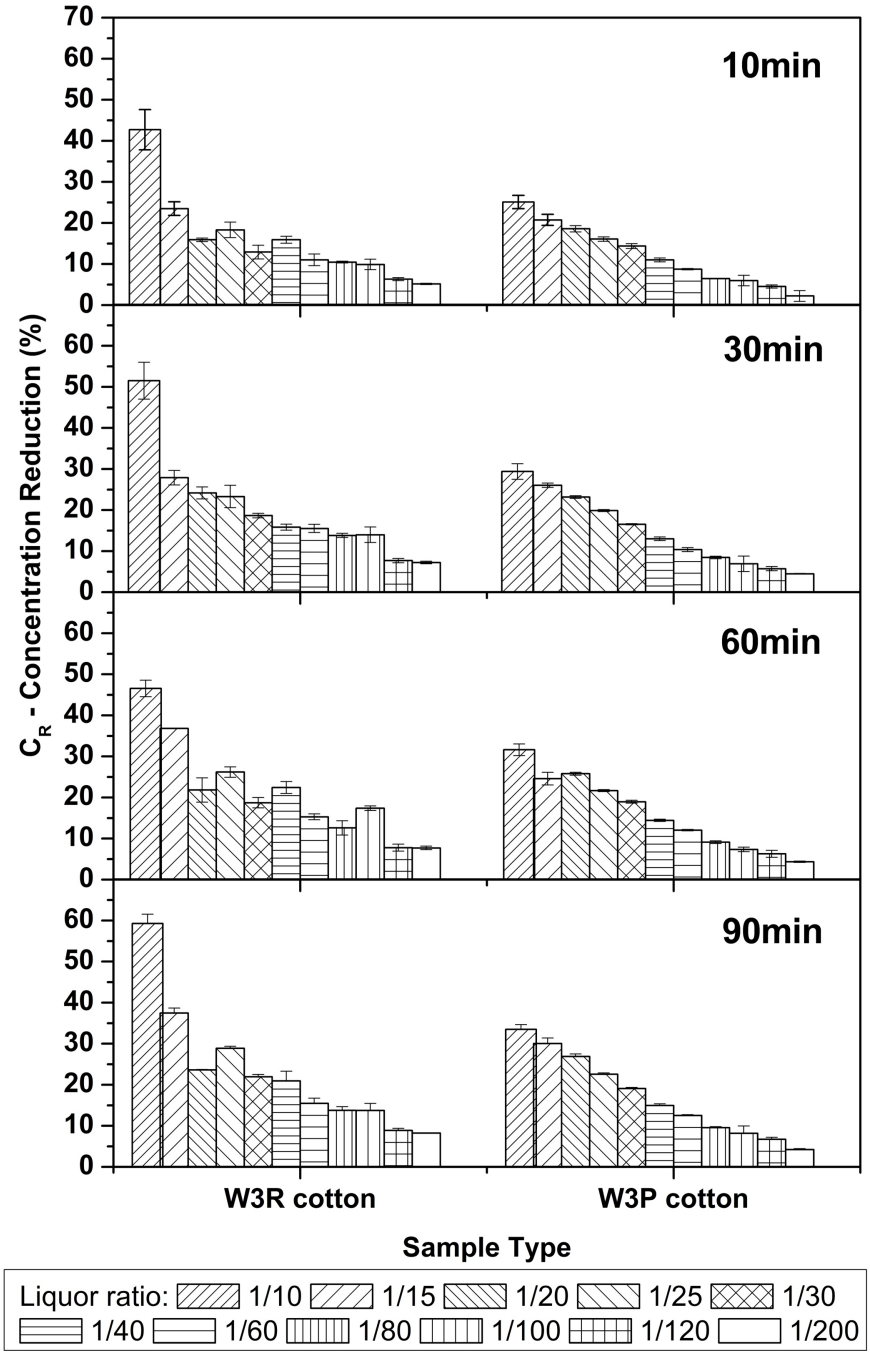


Figure 6. Concentration Reduction (C_R) ± SD of untreated (R) and plasma-treated (P) W3 samples changing with immersion time and liquor ratio.

4.1.3 Concentration of ADBAC absorbed in the wipe (C_Q)

The concentration of ADBAC active ingredients adsorbed in the wipe samples (**C_Q**) is calculated based on Equation 3, $\boldsymbol{C_Q} = C_0 \times \frac{V_0}{V_W} - C_t \times (\frac{V_0}{V_W} - 1)$, in Chapter 3 Materials and Methods. Liquor ratio is expressed in the formula as **M_w / V_0** in this study. The ratio (**V_w / M_w**) between the volume of the ADBAC solution absorbed by the wipe (**V_w**) and the mass of the wipe (**M_w**) representing the material's water absorbency ability, is a fixed value to the same type of material (as discussed in the absorption section). Consequently, the ratio between **V_0** to **V_w** can also be interpreted as a value only depended on the value of **V_0 / M_w** (in reciprocal function of the liquor ratio **M_w / V_0)** for each type of material. Thus, the factors: **C_0** the initial concentration of ADBAC solution, **V_0 / V_w** depending on the material water absorbency or liquor ratio when it comes to the same type of material and **C_t** the remaining concentration of ADBAC solution after removing the wipe, have a positive impact on the final value of **C_Q**. Thus, it can be concluded that, the smaller the liquor ratio is, the higher the concentration of ADBAC in the immersed wipe is.

The **C_Q** results of both untreated and plasma-treated wipe samples vary with liquor ratio (Figure S13, Figure S14, Figure S15in the supporting information). In all W1 wipes no significant adsorption of ADBAC was observed. In this case, the textile substrate is acting as a vehicle that only transfer the ADBAC solution from the stock to the surface applied, thus in equation 3 can be assumed that **C_0** = **C_t**. In untreated and plasma-treated W2 and W3, the **C_Q** is generally increasing with the decrease of the liquor ratio. The plasma-treated wipe samples showed slightly lower values of **C_Q** than untreated ones as previously observed for **C_R**.

4.1.4 Weight ratio between the amount of ADBAC on the wipe and the wipe mass (R_W)

The weight ratio between the amount of ADBAC on the wipe and the wipe mass (**R_W**) can be calculated using Equation 4, $\boldsymbol{R_W} = C_0 \times \frac{V_0}{M_W} - C_t \times \left(\frac{V_0}{M_W} - \frac{V_W}{M_W}\right)$, in Chapter 3 Materials and Methods. This ratio was calculated because different wipes may show the same **C_Q** value but, if the wiping material presents a higher water absorbency property (**V_W**) it will consequently display a larger weight ratio (**R_W**) of active ingredients in the wiping material allowing the wipe application on a larger surface area or for longer wiping time. In W1, **R_W** displayed quasi-constant values independently of the used liquor ratio due to the very low adsorption of ADBAC (Figure S16 in the supporting information) that is dependent on **C_0** and the material type of the wipe. However, the **R_W** of W2 and W3 increased with the diminishing

of the liquor ratio (Figure S17 and Figure S18 in the supporting information). As expected, W3 shows in general lower $\mathbf{R_w}$ than W2 due to its poorer water absorbency properties. Comparing control and plasma, it is noticed that $\mathbf{R_w}$ is very similar in higher liquor ratio (e.g. 1/10, 1/15 and 1/20) in both W2 and W3 wipes, but plasma-treated samples in lower liquor ratios display a diminishing $\mathbf{R_w}$. This phenomenon can be explained by the plasma effect on cellulose that reduce the water absorbency of the wipes. However, this is only noticed in the lower liquor ratios because of the higher quantity of ADBAC that is available in the lower liquor ratios. In higher liquor ratio in which the wipe adsorbs almost all the available volume, and subsequently almost all the ADBAC mass in solution, the phenomenon are therefore less pronounced.

4.2. Chemical interaction analysis

ADBAC is a cationic surface-active agent with a positively charged nitrogen covalently bonded to three alkyl group substituents and a benzyl substituent. It is well known as sanitizers, pharmaceutical antiseptic, medicinal disinfectants, and germicides as one the most widely used QACs.

The interaction between ADBAC and both untreated and DBD plasma-treated wipe samples was analysed by X-ray photoelectron spectroscopy (XPS). The contact angle, surface free energy, and LSM were also measured to clarify the plasma effect in a macroscale.

4.2.1 Contact angle and surface free energy measurement

The results of stationary contact angle, surface free energy and work of adhesion of both untreated wipe samples (R) and plasma-treated wipe samples (P) are displayed in Table 12. W1R (PET) shows hydrophobic behaviour with a contact angle of 139°. All of the other wipes including the plasma-treated ones showed hydrophilic property with a contact angle of 0°. The improved hydrophilicity after the plasma treatment is due to the newly formed polar functional groups on the polyester surface, and the increased surface roughness providing higher surface area for adsorption [208]. This hypothesis is validated by the enhanced polar component value of the plasma-treated wipe (W1P) that increased from 0.68 to 68.14 mJ m^{-2}. On the contrary, W2 and W3 surface energies and works of adhesion decreased after plasma treatment due to the surface plasma etching effect reaching similar values to W1P [130]. Due to these similar values it is difficult to distinguish the effect of cotton and polyester components in

W2P. In W3P, the plasma treatment acts on the micro-fibrillated morphology of the cellulose on the top surface layer of the wipes, reducing the surface area for water absorption by eroding the amorphous regions of the cellulose and maintaining the crystalline domains [209].

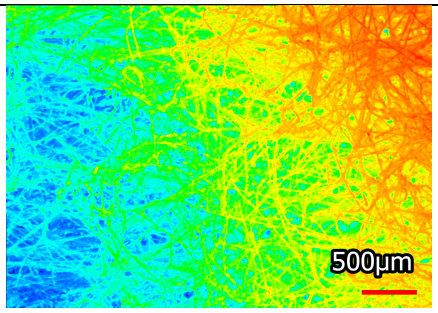
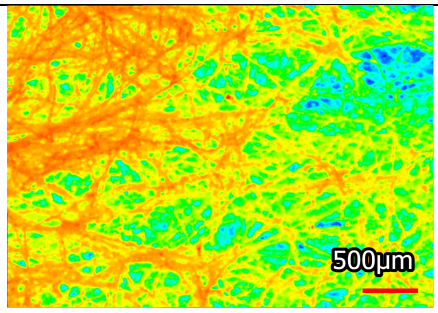
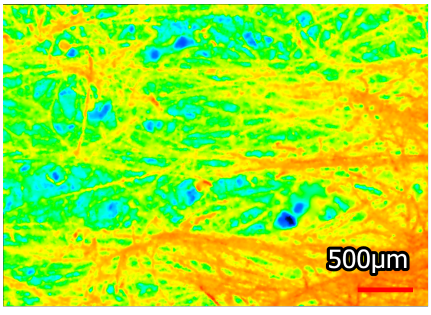
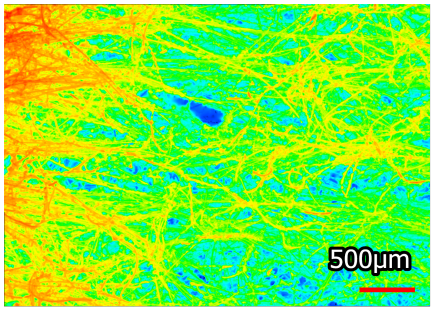
Table 12. Stationary contact angle, surface free energy and work of adhesion of (raw and plasma-treated) wipe samples. Data represent mean values ± SD (n=5).

Sample	$\vartheta_{\text{Distilled water}}$	$\vartheta_{\text{Polyethylene glycol}}$	$\vartheta_{\text{glycerol}}$	Surface energy (mJ m^{-2})	Dispersive (mJ m^{-2})	Polar (mJ m^{-2})	Work of adhesion (mJ m^{-2})
W1R	139.1 ± 3.6	27.9 ± 10.0	140.1 ± 8.0	11.58	10.90	0.68	19.05
W2R	0	22.7 ± 9.9	118.3 ± 7.1	113.22	0.27	112.95	226.44
W3R	0	58.4 ±7.1	47.7 ± 8.1	123.49	5.53	117.96	246.98
W1P	0	0	42.4 ± 7.9	81.84	13.70	68.14	163.68
W2P	0	0	27.9 ± 4.7	80.14	16.50	63.64	160.28
W3P	0	0	40.4 ± 5.8	81.56	14.09	67.48	163.12

4.2.2 Laser scanning microscopy (LSM)

The 3D surface roughness of the wipes before and after plasma treatment was evaluated by laser scanning microscopy (LSM). Figure 7 displays the colour-coded height representations of the surface topography of the wipes (red and blue represent the highest and lowest peaks and valleys, respectively). It is evident that W1 and W2 present differences between the back and front side due to the one-side hydroentangling fabrication method in which a high-pressure water jet produced large spaced nonwovens structures with high and low density regions. W1 structure is more uniform because exclusively composed of thin polyester fibres while W2 clearly shows the entangled structures of the cellulose component in the backside. W3 did not show any difference in both side (data not shown) and the LSM image clearly shows the woven structure of the wipe with few fibres protruding from the surface. After plasma treatment, the surfaces seem more uniformed, which could be a result of the plasma erosive effect (optical and 3D image can be observed in Figure S19 in the supporting information). Table 13 shows the corresponding roughness parameters for the wipes 1, 2 and 3 before

and after plasma treatment in both sides for the nonwovens wipes (W1 and W2) and just in one side for the woven wipe (W3) since its side structure does not present differences. For the nonwoven wipes (W1 and W2) the arithmetical mean height (Sa) and the maximum height (Sz) values are higher in the front sides. These parameters are significantly affected by any surface variability including holes and protruding fibres and provide a measure of the average roughness. After plasma treatment, Sa and Sz values increase in the nonwoven wipes except for the W1 in the front side. It seems that plasma was able to increase roughness due to the erosion of the nonwoven surface structure creating sharper and deeper valleys. This is also confirmed by the hybrid parameters arithmetic mean peak curvature (Spc) and developed interfacial area ratio (Sdr) that increase after plasma treatment. A larger value of Spc indicates pointed peaks on the contact surface while Sdr, expressed as the percentage of the additional surface area contributed by the texture as compared to an ideal plane (Sdr=0 in a flat surface), implies an increase of the surface complexity. The results of the cotton woven wipe after plasma treatment (WP3) showed opposite behaviour decreasing in all the parameters suggesting a smoother surface by plasma etching effect, removing the more hydrophilic surface microfibrils. This is in accordance with the observed reduced water absorbency of WP3 and reinforces the hypothesis that its primary cause is the removal of the micro-fibrillated cellulose on the fabric surface due to plasma etching.

Sample	**Laser Scanning Microscopic Image of heights**	
	Front	**Back**
W1R		
W1P		

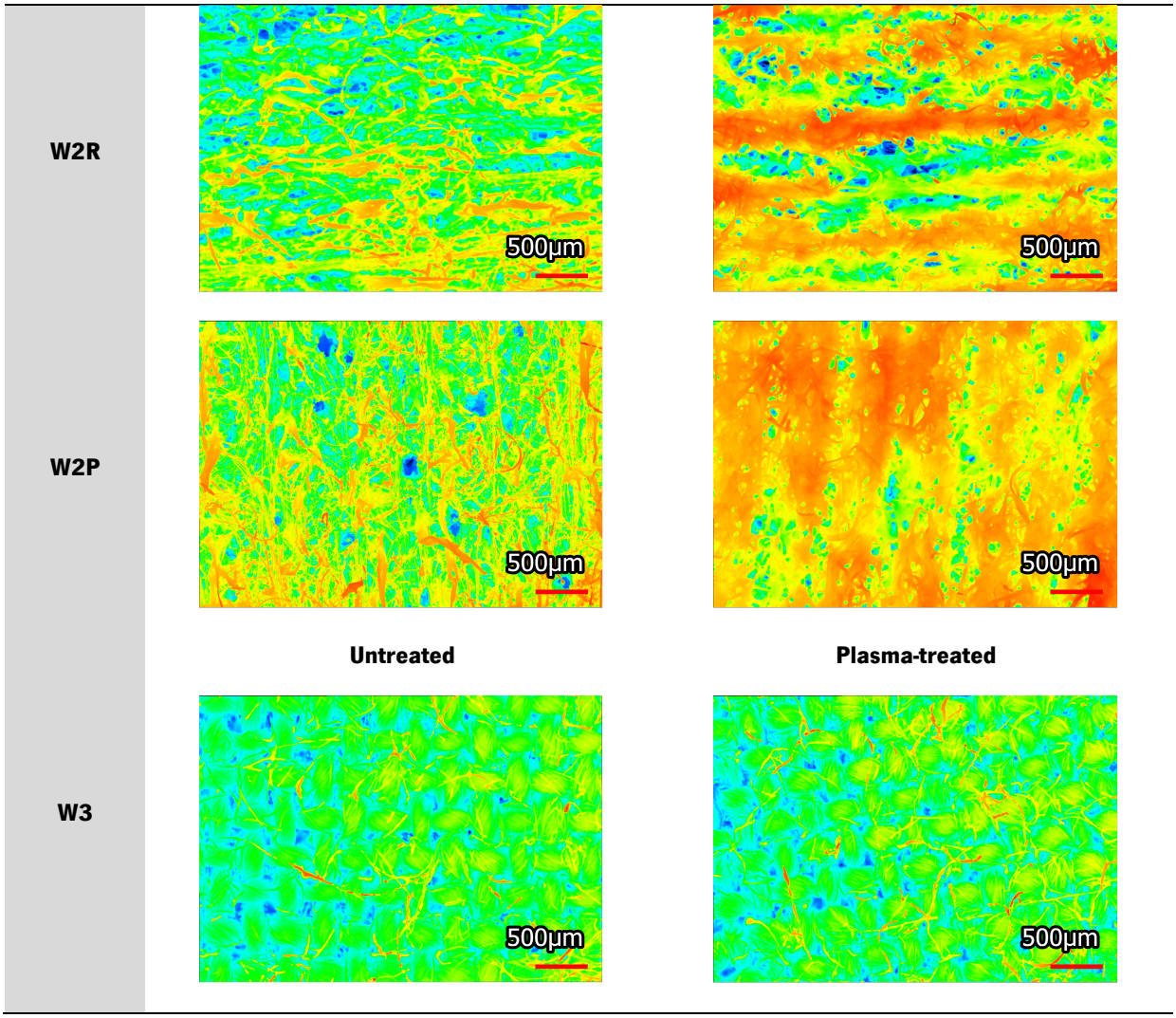


Figure 7. Laser Scanning Microscopic Images of the untreated (R) and plasma-treated (P) wipes in both sides (only for nonwovens). The coloration represents different heights from a theoretical plane in the middle of the sample.

Table 13. Laser scanning microscope results of the untreated (R) and plasma-treated (P) wipes with analysed surface area of 10 mm^2.

Sample	Sa - Arithmetical mean height (µm)	Sz - Maximum height (µm)	Spc - Arithmetic mean peak curvature (mm^{-1})	Sdr - Developed interfacial area ratio (%)
W1R front	78.2	580.5	2255.9	7.9
W1R back	34.1	358.9	1548.7	1.7
W1P front	45.5	510.6	2614.9	10.7

W1P back	37.8	427.9	1563.8	1.7
W2R front	41.7	457.6	1267.2	5.1
W2R back	23.7	206.6	1051.1	2.0
W2P front	47.0	540.3	3161.5	20.1
W2P back	24.5	330.9	1085.1	2.3
W3R	31.5	568.5	1842.2	6.7
W3P	25.9	415.0	1560.7	4.9

4.2.3 XPS analysis

The wipe samples selected for XPS analysis are the ones with liquor ratio 1/20 and 90 minutes of immersion time in ADBAC in order to reach the equilibrium in the ADBAC adsorption. The degree of chemical modifications on the surface of the wipes was studied by XPS (Figure S20, Figure S21, and Figure S22 for XPS survey scan can be found in the supporting information). The relative chemical composition (C, N, O) and oxygen and nitrogen atomic ratios (O/C and N/C) were exhibited in Table 14. Plasma-treated wipes containing polyester (W1 and W2) were significantly altered in terms of oxygen content showing an increase of O/C ratio about 36% and 13% for W1 and W2, respectively. DBD plasma discharge in air at atmospheric pressure is able to generate a wide range of active species such as atomic oxygen, ozone, nitrogen oxides and radicals. After plasma treatment, the considerable increase in the oxygen content is due to the incorporation of oxygen-containing polar groups onto the polyester fibres surface generating hydroxyl and carboxyl groups [210, 211]. W3 shows only a slight increase of about 5% in O/C ratio confirming that this DBD plasma is not able to substantially oxidize the cellulose polymer chain, providing only etching effect. Most of the work demonstrating cotton surface oxidation by atmospheric plasma in the air were developed using raw cotton that contains several non-cellulosic components in cuticle and primary wall [195, 212]. However, in this work, a white bleached woven cotton was used preventing further surface oxidation. The observed residual nitrogen component in the untreated control wipes is due to the adsorbed N from the atmosphere since none of the used wipes polymers contains nitrogen in its structure. After plasma treatment, the increase in N component in these wipes is due to the incorporation of atmospheric nitrogen to the fabric surface during the plasma reactions as previously reported [213, 214]. However, all the wipes did not show any significant difference in the N/C ratio.

After the introduction of ADBAC, the nitrogen content increased as expected since every ADBAC molecule contains a nitrogen atom. Polyester-based W1 wipe (W1Q) showed the higher amount of nitrogen content (1.65%). This is due to the higher hydrophobicity of pure polyester compared to the other wipe materials which hamper ADBAC solution to be absorbed in the bulk of the wipe resulting in higher ADBAC concentration on the wipe surface. However, plasma-treated W1Q shows a higher N/C atomic ratio comparing with the untreated W1Q (about 50%). This suggests that a significant part of the adsorbed ADBAC remains on the surface of the polyester and after plasma treatment, its surface concentration increases even more, reacting with the newly formed oxygen species. For W2 sample, a slight increase around 9% growth in the nitrogen content can be observed. This is due to the blended composition of polyester and cellulose. Since the cellulose fibres are only etched and adsorb the ADBAC in its inner structure, in W2, the increase in oxygen and nitrogen content can be mainly attributed to the plasma-treated polyester component and the ADBAC in its surface, respectively. A slight difference in the nitrogen content between untreated and plasma-treated W3 (28% reduction from 0.90 to 0.65) was observed. This is in accordance with the LSM results showing a cotton surface roughness reduction (Table 13). It was previously observed that the etching of the cotton surface microfibrils is able to promote the reorientation of the polar surface functional groups reducing the active ingredient adsorption [215].

Table 14. Relative chemical composition and atomic ratio of untreated and DBD plasma-treated water/ADBAC immersed wipe samples result from XPS analysis.

Sample	**Untreated**					**Plasma-treated**				
	Chemical composition (%)			**Atomic ratio**		**Chemical composition (%)**			**Atomic ratio**	
	C	**O**	**N**	**O/C**	**N/C**	**C**	**O**	**N**	**O/C**	**N/C**
W1	73.10	26.35	0.60	0.36	0.01	65.85	32.55	0.75	0.49	0.01
W2	68.90	30.65	0.40	0.45	0.01	65.75	33.25	1.05	0.51	0.02
W3	61.70	37.80	0.50	0.61	0.01	60.65	38.8	0.55	0.64	0.01
W1Q	82.70	14.65	1.65	0.18	0.02	79.95	18.00	2.00	0.23	0.03
W2Q	61.30	38.15	0.55	0.62	0.01	61.85	37.55	0.60	0.61	0.01
W3Q	63.50	35.55	0.90	0.56	0.01	62.80	36.60	0.65	0.58	0.01

The deconvolution result of the high-resolution XPS spectra of C1s, O1s, and N1s was summarized in Table 15. All the deconvolution graphs can be found in Figure 8 (W1), Figure 9 (W2), and Figure 10 (W3). For W1, of 100% polyester, the C1s envelops of both untreated and plasma-treated samples were deconvoluted into 3 peaks attributed to C-C or C=C or C-H (284.8 eV), C-O or C-O-C (286.5 eV), and O-C=O (288.8 eV) [216]. After plasma treatment, there was a slight change of the bonding distribution (increasing of the carbon-oxygen intensity) due to the inclusion of oxygen species from the atmosphere (Figure 8). This was also confirmed from the result of O1s where the envelope can be deconvoluted in two peaks at 532 eV and 533.3 eV attributed to the carbonyl oxygen and the oxygen atoms single bonded to carbon atoms of the PET structure, respectively [217, 218]. After plasma treatment, an increase in the carbonyl oxygen can be noted. After the introduction of the ADBAC, there was a negative shift of C-C binding energy (from 284.8 eV to 284.4 eV) as well as an increase in the peak relative percentage (W1R from 68.3% to 79.2%, and W1P from 58.8% to 76.6%), resulting from the increased amount of phenyl aromatic group and long alkyl group chain of ADBAC on the wipe surface [219, 220]. In the W1RQ sample, the new peak at 285.7 eV is attributed to the C-N bond of ADBAC confirming its presence on the surface of the W1 [221]. The peak attributed to the O-C=O group was significantly reduced (from 12% to 6%) due to the introduction of the long alkyl chain of ADBAC, and shifted to lower binding energies at 288.5 eV suggesting an interaction of ADBAC molecule with the oxygen species on the polyester surface. The plasma-treated surface of W1PQ showed similar binding energy and intensity for the C-C and O-C=O components to the untreated sample W1RQ. However, the significant shift of the C–N bond to higher binding energy (286.2 eV) suggested a strong interaction of the ADBAC molecule with the oxygen single bonded to carbon. The new peak at 530.8 eV, appearing in the O1s deconvolution result of both W1RQ and W1PQ, may be attributed to the ADBAC oxidation and interaction with the polyester [222, 223]. After plasma treatment, these peaks increased from 24.8% to 29.9% due to the ADBAC surface interaction with the newly formed polyester oxygen species. The confirmation of the surface deposition of ADBAC onto the W1 surface comes to the deconvolution of the N1s high-resolution spectra (Figure 8). Among all the analysed wipes, only W1PQ (plasma-treated pure polyester with ADBAC) showed peaks with intensities well above the detection limits. Moreover, only this sample can be deconvoluted in two well distinct peaks at 405.8 eV and 401.8 eV. The peak at 401.8 eV can be assigned to the N-C bonding of the ADBAC structure while the peak at 405.8 eV is associated to the nitro group confirm the strong interaction of the ADBAC with the oxygen species on the polyester surface [224, 225]. All other wipes containing ADBAC showed only a very faint peak, in the edge of the

detection limit, associated to the ADBAC structure at 401.8 eV indicating that only the plasma-treated W1 retains a significant amount of ADBAC onto the very surface of the wipe interacting with the newly generated oxygen species on polyester.

For W2, that is a mix of polyester and cellulose, the C1s spectra of both untreated and plasma-treated wipe samples were deconvoluted at the same peak positions of W1 (Figure 9). Also in this case, after plasma treatment, an increase of the carbons bonded to oxygen species was observed. However, the peak at 286.2 eV attributed to the oxygen atoms single bonded to carbon atoms show a significantly higher relative percentage compared to W1 due to the presence of cellulose. Deconvolution of the O1s core level of W2R consists of two peaks. The first at 532.8 eV is attributed to the single-bonded oxygen of the ester functional group (O=C-O) of polyester and C–OH from cellulose [226, 227]. The second, at 531.5 eV is assigned to the O–C–O/C–O functional group of cellulose and to the double-bonded oxygen of polyester (O=C-O) [228]. After plasma treatment, W2P displayed a shift of the peaks to higher binding energies due to the increase of the oxidation state on the wipe surface. The introduction of ADBAC in W2 radically change the peak intensities showing the typical C-C (284.8 eV), C-O (286.5 eV) and O-C-O (287.8 eV) components and relative percentages of cellulose [229]. It seems that the adsorption of ADBAC addition promotes the swelling of the cellulose component of the wipe W2 resulting in the predominant species on the wipe surface during the XPS analysis. This is also confirmed by the absence of significant nitrogen component on the wipe surface. However, the O1s deconvolution showed a dominant peak at 532.8 eV in both W2RQ and W2PQ attributable to the single-bonded oxygen of cellulose. The component at 530.8 eV of the ADBAC interaction with the oxygen species on polyester fibres resulted in only about 5% of the O1s envelope deconvolution confirming that most of the disinfectant is adsorbed by the cellulose component and is not available on the wipe surface.

The woven W3 wipe composed exclusively of bleached cotton showed the typical C1s spectra of cellulose (Figure 10). C1s envelope was deconvoluted for all the samples into the same three peaks at 284.8 eV, 286.5 eV, and 287.8 eV corresponding to the C-C, C-O and O-C-O components of cellulose [230]. The deconvolution of the O1s high-resolution spectra of the W3 wipes displays the two typical peaks of cellulose at 531.8 eV and 533.3 eV except for W3PQ that show a shift to lower binding energies at 531.5 eV and 532.8 eV [231]. This shift could be attributed to the swelled and lower oxidized plasma-treated cotton surface after ADBAC adsorption [232].

Table 15. Results of the deconvolution analysis of the C1s, N1s, and O1s peaks for the untreated (R) and DBD plasma-treated (P) wipes before and after ADBAC adsorption. Reported binding energies have an associated error of ± 0.3 eV.

	Relative area corresponding to different chemical bonds (%)															
Sample	**C1s**								**O1s**						**N1s**	
	284.4 eV	**284.8 eV**	**285.7 eV**	**286.2 eV**	**286.5 eV**	**287.8 eV**	**288.5 eV**	**288.8 eV**	**530.8 eV**	**531.5 eV**	**531.8 eV**	**532.0 eV**	**532.8 eV**	**533.3 eV**	**405.8 eV**	**401.8 eV**
W1R	-	68.3	-	-	19.4	-	-	12.3	-	-	-	49.1	-	50.9	-	-
W1P	-	58.8	-	-	22.3	-	-	18.9	-	-	-	50.5	-	49.5	-	-
W1RQ	79.2	-	15.1	-	-	-	5.8	-	24.8	-	-	43.1	-	32.1	-	100*
W1PQ	76.6	-	-	17.5	-	-	5.9	-	29.9	-	-	43.7	-	26.4	35.4	64.6
W2R	-	56.7	-	-	28.1	-	-	15.2	-	36.0	-	-	64.0	-	-	-
W2P	-	50.7	-	-	29.7	-	-	19.6	-	-	41.1	-	-	58.9	-	-
W2RQ	-	17.2	-	-	67.6	15.2	-	-	4.4	-	-	-	95.6	-	-	100*
W2PQ	-	29.7	-		57.2	13.1	-	-	5.9	-	-	-	94.1	-	-	100*
W3R	-	27.7	-	-	60.2	12.1	-	-	-	-	28.8	-	-	71.2	-	-
W3P	-	24.9	-	-	64.9	10.2	-	-	-	-	33.1	-	-	66.9	-	-
W3RQ	-	28.5	-	-	59.4	12.1	-	-	-	-	16.1	-	-	83.9	-	100*
W3PQ	-	35.1	-	-	54.2	10.7	-	-	-	37.7	-	-	62.3	-	-	100*

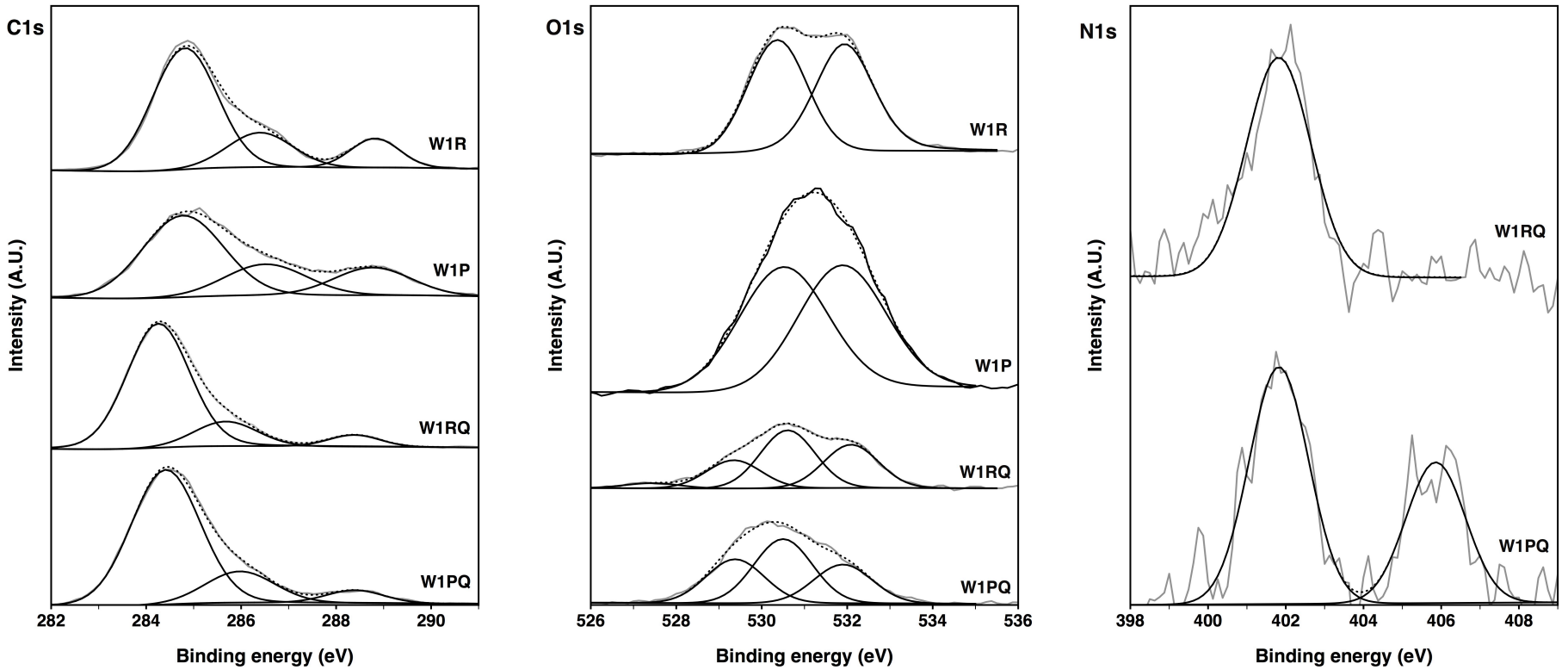


Figure 8. High-resolution XPS spectra deconvolution of the C1s, O1s and N1s binding energy regions of wipes 1 (W1) for the untreated (R) and DBD plasma-treated (P) wipes before and after ADBAC adsorption.

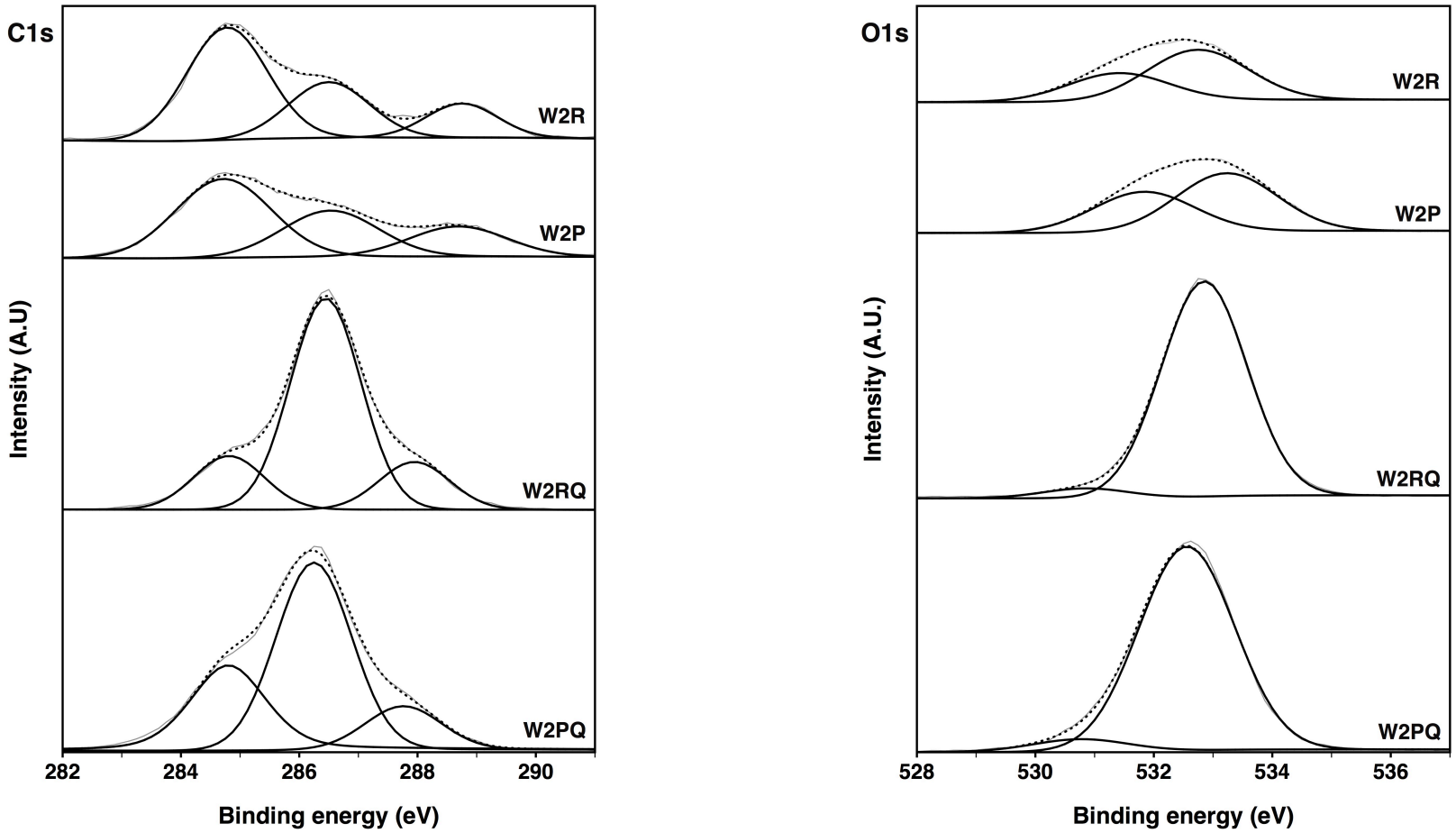


Figure 9. High-resolution XPS spectra deconvolution of the C1s and O1s binding energy regions of wipes 2 (W2) for the untreated (R) and DBD plasma-treated (P) wipes before and after ADBAC adsorption.

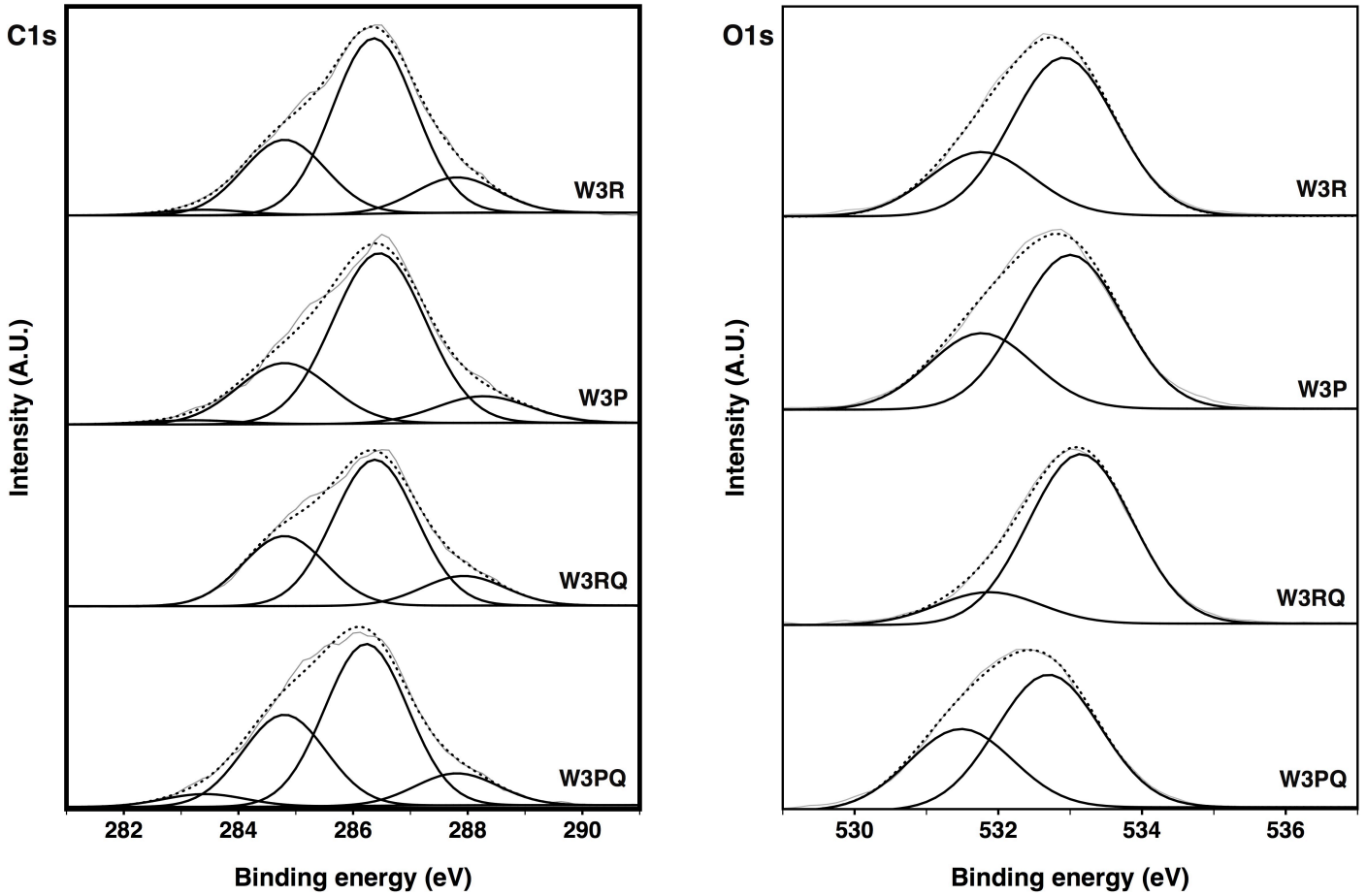


Figure 10. High-resolution XPS spectra deconvolution of the C1s and O1s binding energy regions of wipes 3 (W3) for the untreated (R) and DBD plasma-treated (P) wipes before and after ADBAC adsorption.

4.3. Ageing performance

The ageing of the untreated and dielectric barrier discharge (DBD) plasma-treated disinfectant-containing wipes after 30 min, 3 days, 7 days, 15 days and 30 days of storage time was studied in terms of structure, function, chemical and thermo-mechanical properties change. The concentration reduction of bulk ADBAC solution before and after wipe immersion at the different aged time was analysed by UV-Vis spectrophotometry. Fourier-transform infrared spectroscopy (FTIR) was used to study the chemical change of the wipe surface and Dynamic mechanical analysis (DMA) was used to evaluate the thermo-mechanical properties change of the wipe materials for 7 days storage time. Breaking force and elongation change were also recorded with a universal testing machine (UTM).

4.3.1 Adsorption of ADBAC during storage time

Figure 11 exhibits the concentration change of bulk ADBAC solution during storage time up to 30 days. For W1 (100% PET), both untreated and plasma-treated wipes showed extreme low adsorption (expressed as the percentage of concentration reduction in Figure 11). However, the plasma-treated wipe showed a slightly higher adsorption than the untreated one. This can be explained by the plasma-induced increase of surface energy polar component by surface oxidation (as also observed by the fibre yellowing), which can enhance the interaction between polyester and ADBAC molecules [191]. Plasma treatment can generate active oxygen species on the polyester surface, such as hydroxyl groups, providing partially negative charge on the surface which can interact with the positively charged ADBAC molecules [233]. Meanwhile, the increased hydrophilicity of polyester samples improved the absorption of ADBAC solution on the textile substrate (Table 12). Due to these two reasons, which was previously confirmed by XPS analysis, the adsorption increased. Plasma-treated polyester sample displayed an evident increase in the adsorption up to 7 days, then it stabilized. Even though polyester samples showed an increase after plasma, its adsorption remains the lowest among the studied wipes.

W2 (CEL/PET) shows a medium adsorption because of the negatively charged cellulose fibres interacting with the positively charged ADBAC active ingredient promoting adsorption. After plasma treatment, W2 wipes duplicated the adsorption ability (from 15% to about 30%) by the mutual action of the plasma oxidation of polyester fibres and by the already hydrophilic cellulose component. It seems that plasma treatment is able to enhance the cellulose absorption ability of W2 by increasing the liquid

mass transfer into the inner part of the wipe. The swelling effect becomes more pronounced after plasma treatment facilitated by the shift of the cellulose shear plane towards the solution and furthermore increasing the adsorption [196, 234].

W3 is a 100% cellulose wipe exhibiting the highest adsorption ability among all the wipe samples. However, plasma-treated W3 showed a decrease in adsorption compared to its control. On one hand, in literature, plasma treatment is efficiently used to clean raw cotton by removing its non-cellulosic components such as waxes, proteins and pectin, oxidizing its surface and increasing polar functional groups [195, 235]. On the other hand, in this case, the used wipe was a white bleached cotton woven fabric that is already chemically oxidized as proved by XPS analysis (Table 14). Plasma treatment reduced the surface roughness (LSM results in Table 13) by etching the microfibrils on the cotton surface and promoted reorientation of the polar surface functional groups which significantly reduced the adsorption of ADBAC of around 10% [215].

Taking into account the XPS surface analysis result, it is noted that even though there is large adsorption of ADBAC in W2 and W3 (Figure 11), more ADBAC can be detected on the surface of W1 (Table 14), indicating that the absorbed ADBAC is more present on the surface of W1 comparing with the other two.

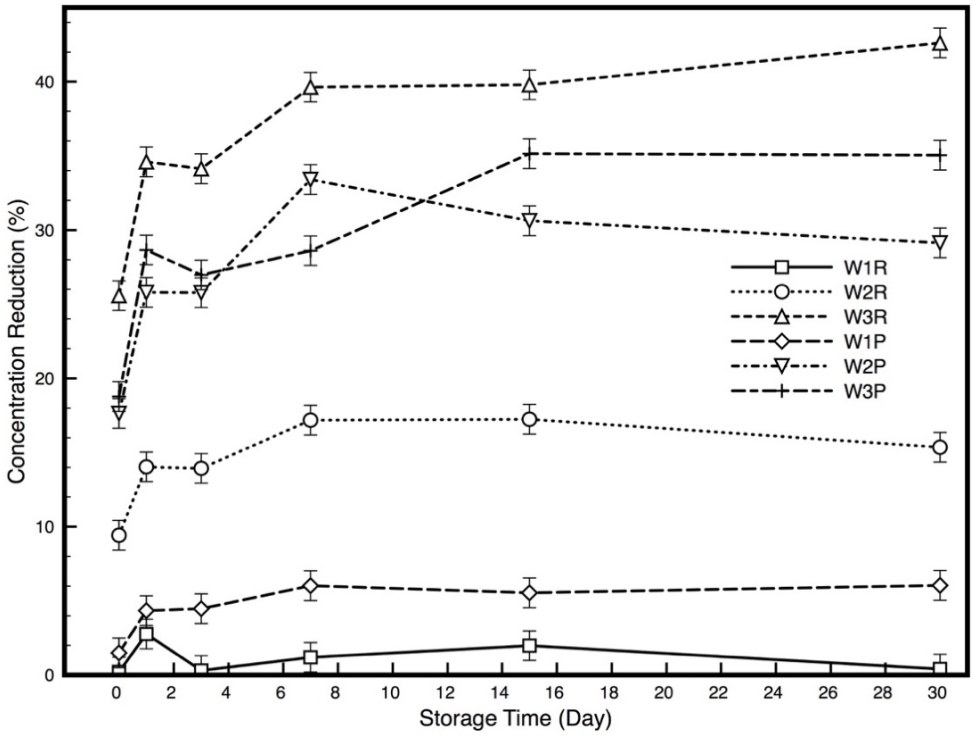


Figure 11. Concentration reduction in ADBAC bulk solution during storage time.

4.3.2 Breaking force and elongation at break over storage time

Wipes used for disinfection purpose should exhibit high tear resistance and tensile strength, low elongation and good abrasion resistance allowing even heavy dirt removal without fibre shedding or breaking and size deformation in the cleaning process. Breaking force and elongation at break of the wipe samples were tested in cross direction (CD) and machine direction (MD) for nonwovens (W1 and W2) and in warp and weft directions for woven structure (W3). As expected, the breaking force is greater in machine direction than in the cross direction in both nonwoven wipe samples (Figure 12). W1 showed double breaking force in CD direction than W2 attributable to the wipes production feature. After plasma treatment, a slight decrease in the breaking force is noted but with no significant changes during ageing. In W3 the ADBAC seems to have a small effect in increasing the force during the time up to 7 days compared to water. Plasma treatment clearly has an effect in weft direction reducing the force at break in water and ADBAC treated wipes of about 26% and 33%, respectively. No significant difference can be noted in the warp direction. This decrease in breaking force can be explained by the reduction in inter-fibre friction after plasma treatment. As observed by LSM, inter-fibre frictional forces of the plasma-treated W3 wipe decreased by the smothering of the cotton fibre surface by etching. Lower forces are needed to overcome the decreased inter-fibre friction resulting in lower breakage loads [236, 237].

Elongation at break is larger in CD than in the MD for all the nonwoven wipes (Figure 13). The plasma-treated wipes showed a slight decrease in CD but not in MD directions. Control W3 shows a clear decrease in elongation in function of the storage time in water after 7 days and in ADBAC after 3 days. The storage of wet wipes clearly has a significant effect on the reduction of the elongation due to the swelling of fibres which restrict the movement of the yarns resulting in a significant loss in elongation (8%). As previously observed, quaternary ammonium salts, enhancing fibres swelling and accelerate the ageing of the fibres reducing the time in which the fibres lost their elasticity [238]. Surprisingly, plasma-treated W3 in weft direction did not show any loss but a slight increase in elasticity during storage time. This is in accordance with the previously observed decrease in breaking force in the weft direction. No significant changes can be depicted in the warp direction.

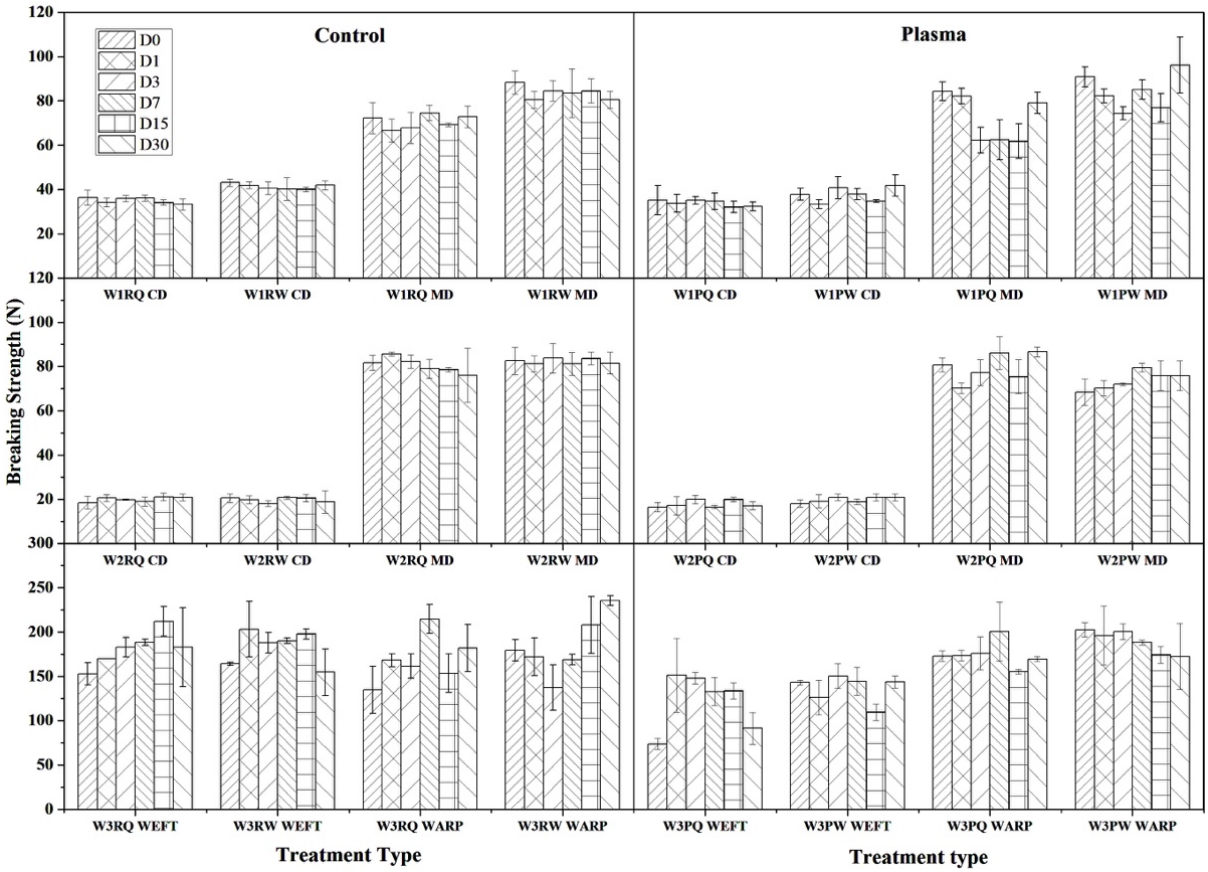


Figure 12. Breaking force (N) change of control (R) and plasma-treated (P) wipe samples during 30 days of storage time (D0 to 30 represented 30 mins, 1, 3, 7, 15, and 30 days' immersion time).

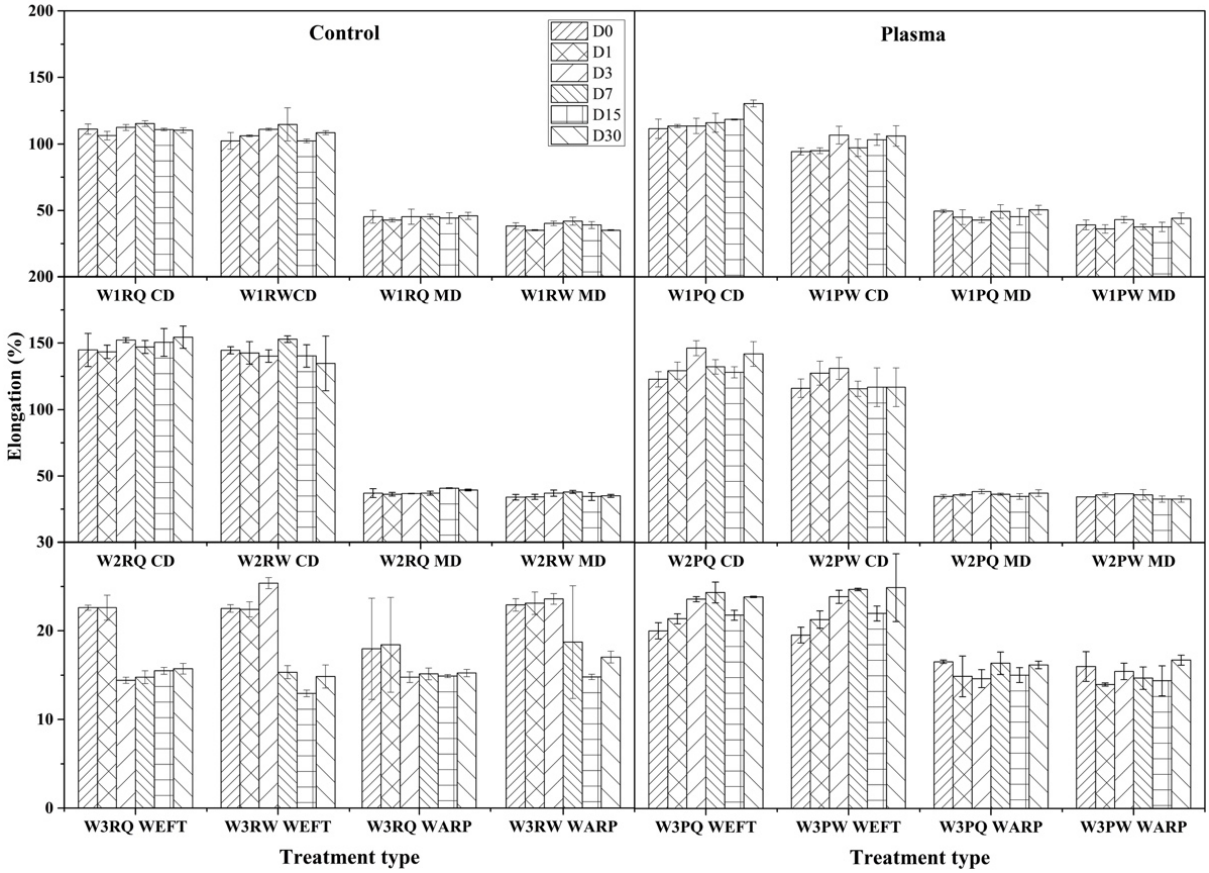


Figure 13. Elongation at break (%) change of control (R) and plasma-treated (P) wipe samples during 30 days of storage time (D0 to 30 represented 30 mins, 1, 3, 7, 15, and 30 days' immersion time).

4.3.3 Chemical change of the wipe surface over storage time (FTIR)

In order to determine the chemical change of the wipe samples over storage time in ADBAC solution, FTIR spectroscopy measurements were performed with untreated, plasma-treated and ADBAC immersed wipe samples. The ATR-FTIR spectrum (Figure 14) of untreated W1 wipe exhibited peak of the polyester at about 1710 cm^{-1} assigned to carbonyl (C=O) stretching vibration in ester, the strong bond at 1250 cm^{-1} assigned to asymmetric stretching of aromatic ester, at 871 cm^{-1} attributed to C–C out of plane bending mode of the benzene rings, and peak near 710 cm^{-1} may be attributed to aromatic C-H bending vibrations [239]. The spectra of W2 and W3 displayed the strong bands at around 2900 cm^{-1} assigned to the symmetric stretching vibrations of C-H attributed to the cellulose structure [196]. The broad and strong bands at 3340 and 3270 cm^{-1} indicated the stretching vibration of the hydroxyl (OH) group of the cellulose structure [240]. The strong peaks at 1150, 1100 and 1020 cm^{-1} are from the vibrations of the C-O-C bond of the glycoside bridges of the cellulose structure [241, 242]. However,

in W2 no peak of the polyester appeared in the FTIR spectra due to the strong intensity of the cellulose peaks covering the polyester ones. The FTIR result did not exhibit any significant change over storage time or plasma treatment for all the tested wipes (Figure S23, Figure S24 and Figure S25 in the supporting information), indicating that FTIR is not sensitive enough in detecting the chemical changes after plasma treatment since penetration depth of FTIR is around 2 µm [243]. FTIR-ATR is not able to detect the small amount of ADBAC on the wipes as well as changes in the non-covalent bonds such as ionic, hydrogen, van der Walls or electrostatic forces that can significantly influence the adsorption of the active ingredient. (Therefore, XPS technique was employed.)

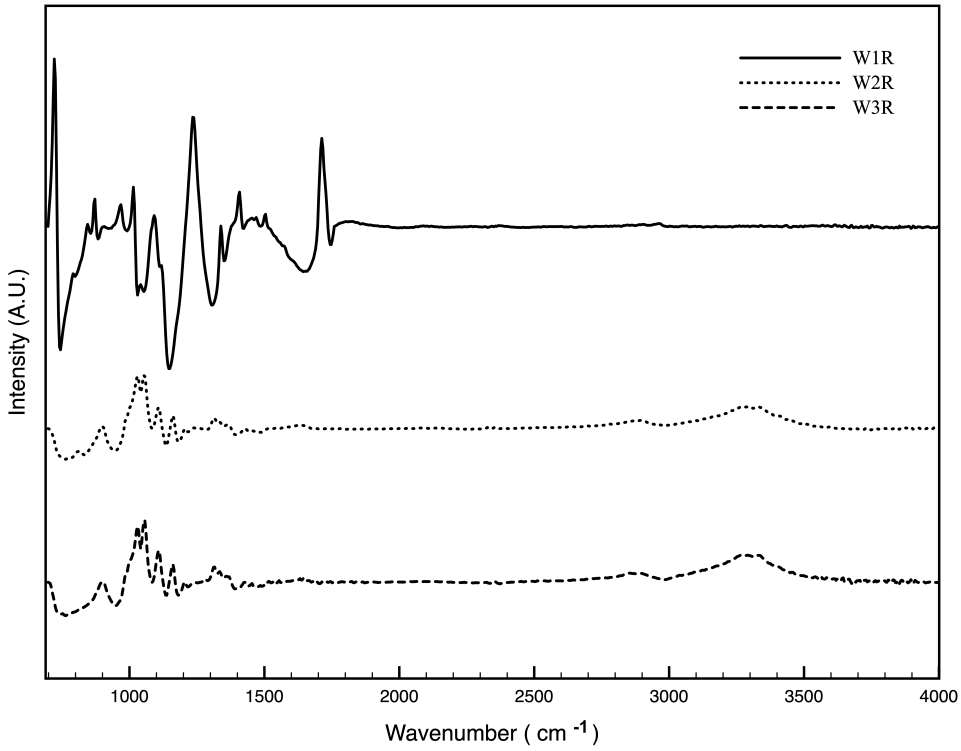


Figure 14. ATR-FTIR spectrum of untreated W1, W2 and W3 samples in the spectral range between 700 and 4000 cm^{-1}.

4.3.4 Dynamic mechanical analysis over storage time (DMA)

The dynamic mechanical analysis (DMA) was performed to study the thermo-mechanical properties change of the wiping materials over storage time without and with plasma treatment. The DMA parameters including tan delta, loss and storage moduli provide important information about the stiffness of the polymer, molecular motion, relaxation process, structural hetero groups, and

morphology of the polymer blend systems [244]. Storage modulus (E') describes the stored energy in the polymer, which reflects the measure of elastic response of a material. While loss modulus (E'') defines the energy dissipated as heat, which represents the plastic response. Figure 15 presented the storage modulus of control wipe samples (R) and their plasma-treated wipe samples (P) at Day 7. W1 (Figure 15-A) control and plasma-treated samples showed significant difference for water and ADBAC immersion treatment. In the untreated wipe sample, the storage modulus at 30 °C increased three times with the addition of ADBAC from 1.3 GPa to 3.8 GPa. Meanwhile, in the plasma-treated wipe sample, the storage modulus increased from 1.7 GPa to 4.5 GPa. The increased storage energy in ADBAC immersed wipe samples showed that the quaternary ammonium salt is able to alter the intermolecular bonding that hinder the mobility of polymer chains in the wipes [245]. This values maintained relatively stable up to 100 °C. After this temperature, all samples storage modulus started to decrease due to the increased mobility of the polymer chains. However, ADBAC samples (untreated and plasma-treated) displayed a higher decrease to values similar to water storage modulus probably due to the rapid ADBAC molecule degradation. Plasma-treated wipe samples gave a higher storage modulus than the control wipe in either ADBAC or water samples. The improvements in the thermo-mechanical properties of the plasma-treated wipes can be associated to the improved adhesion among fibres in the interface region promoted by the plasma generated species such as free radicals and other oxidised functional groups introduced on the surface of the polyester wipes [246]. W2 wipes (Figure 15-B) did not show any significant differences among all the sample behaving like a composite blend showing both the mechanical properties of cellulose and polyester at the same time. Untreated W3 wipes displayed significant differences between ADBAC and water immersed samples due to the interaction between ADBAC and cellulose. The ADBAC immersed wipe sample showed a higher storage energy than the one immersed in water resulting from the electrostatic forces between the quaternary ammonium salt and the cellulose structure [247, 248]. Despite the XPS analysis did not show significant oxidation of the cellulose surface, plasma-treated W3 wipes showed dramatic changes in the thermo-mechanical properties in both water and ADBAC immersed wipes (Figure 15-C). Contrarily to W1, the control W3 wipe exhibits a much higher storage modulus than the plasma-treated one (1 GPa vs 0.3GPa). This change may result from the etching effect of plasma treatment on cotton fabric. The etching effect smoothens the cotton fabric surface by removing crosslinked impurities and surface cellulose microfibrils, as previously discussed, leading to a decrease in storage modulus. Similar

behaviour was observed for all the wipes in the loss modulus (Figure S26 in the supporting information).

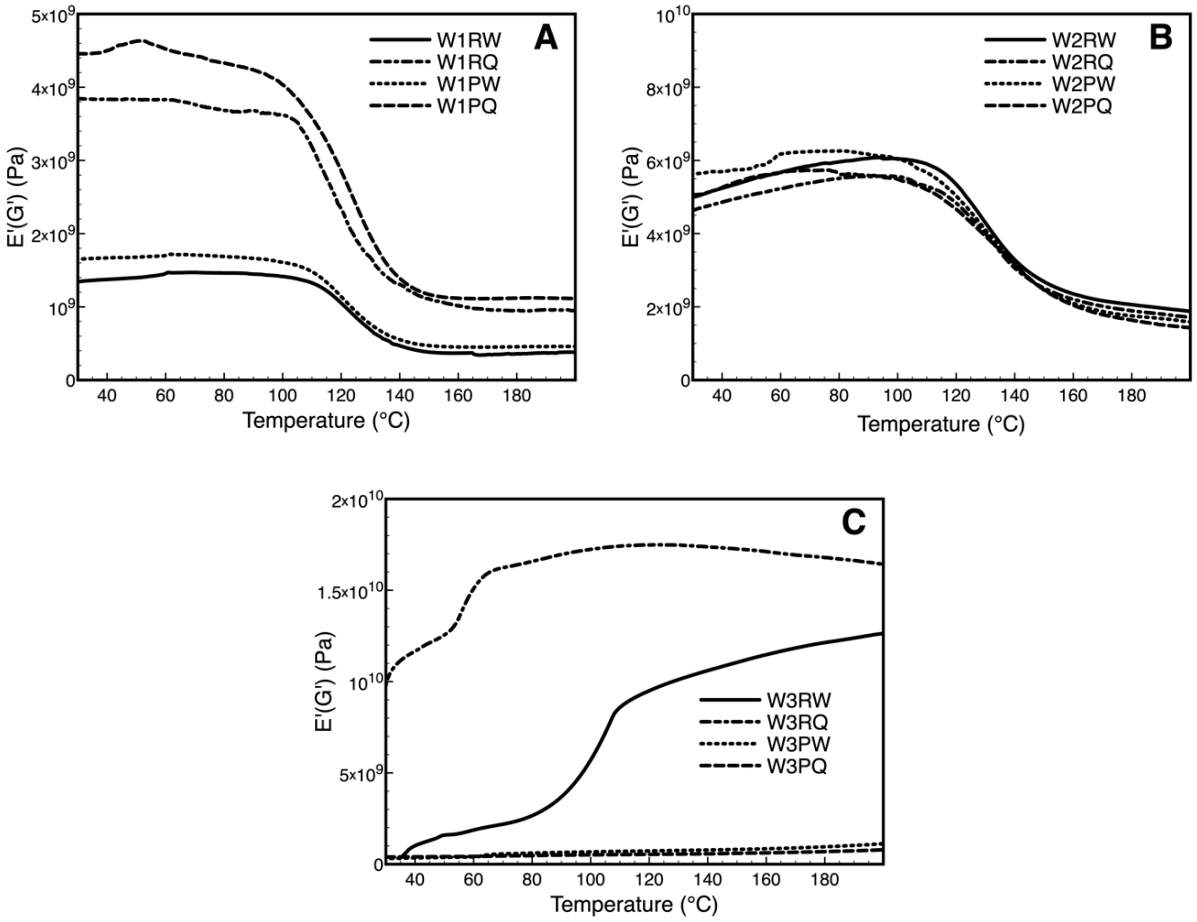


Figure 15. Temperature dependence at 4 Hz of storage (E') modulus of W1 (A), W2 (B), W3 (C) of untreated (R) and plasma-treated samples (P) at Day 7 immersed in water (W) and ADBAC (Q).

The tan delta, also known as the damping factor is the ratio between the loss (E'') and storage (E') modulus and it is an indicator of the molecular motions in a material. A low tan δ value exhibits a material possessing a more elastic strain component, on the contrary, a high value implies a more non-elastic feature. The presence of ADBAC and plasma treatment resulted in a decrease in tan δ in W1 samples (Figure 16-A) due to the stress transfers between wipe and the ADBAC and the plasma introduced functional groups altering the intermolecular bonding that changed the mobility of polymer chains in the wipe. The decrease in the magnitude of tan δ upon addition of ADBAC or plasma treatment to the wipes suggested a limited mobility of polymeric chains of polyester because of the

interactions of the ADBAC or by the plasma-generated functional groups that caused the decrease in damping factor [249]. The glass transition temperature shift of the ADBAC in untreated control to lower temperature confirmed the plasticizing effect of the quaternary ammoniums salt on the polymeric network only when no plasma-generated oxygen species were present [250, 251]. As expected, W2 did not show a significant difference in tan δ values with different treatments suggesting that the overall viscoelastic properties of the blend were not perturbed by the ADBAC immersion and plasma treatment probably due to the absorption/adsorption ability of cellulose compensating the interaction of the ADBAC and plasma-generated species in polyester (Figure 16-B). W3 wipe is clearly the most affected one in its thermo-mechanical properties by ADBAC immersion and plasma treatment (Figure 16-C). Control W3 immersed in water displayed a decrease in tan δ values indicating a more elastic property by the raise of temperature. However, the ADBAC immersed control samples gave a much lower tan δ value due to the interaction between ADBAC and cellulose. It seems that the electrostatic forces interactions enhanced the elastic property of cellulose. However, the influence from the interaction is getting weak by raise of the temperature, especially after 110 °C. Surprisingly, the tan δ values of plasma-treated W3 samples were found to be temperature independent both for water and ADBAC samples. The plasma effect in cotton clearly increased the non-elastic strain component due to the etching effect that removes the crosslinked impurities and the entangled micro-fibrillated structures as previously discussed.

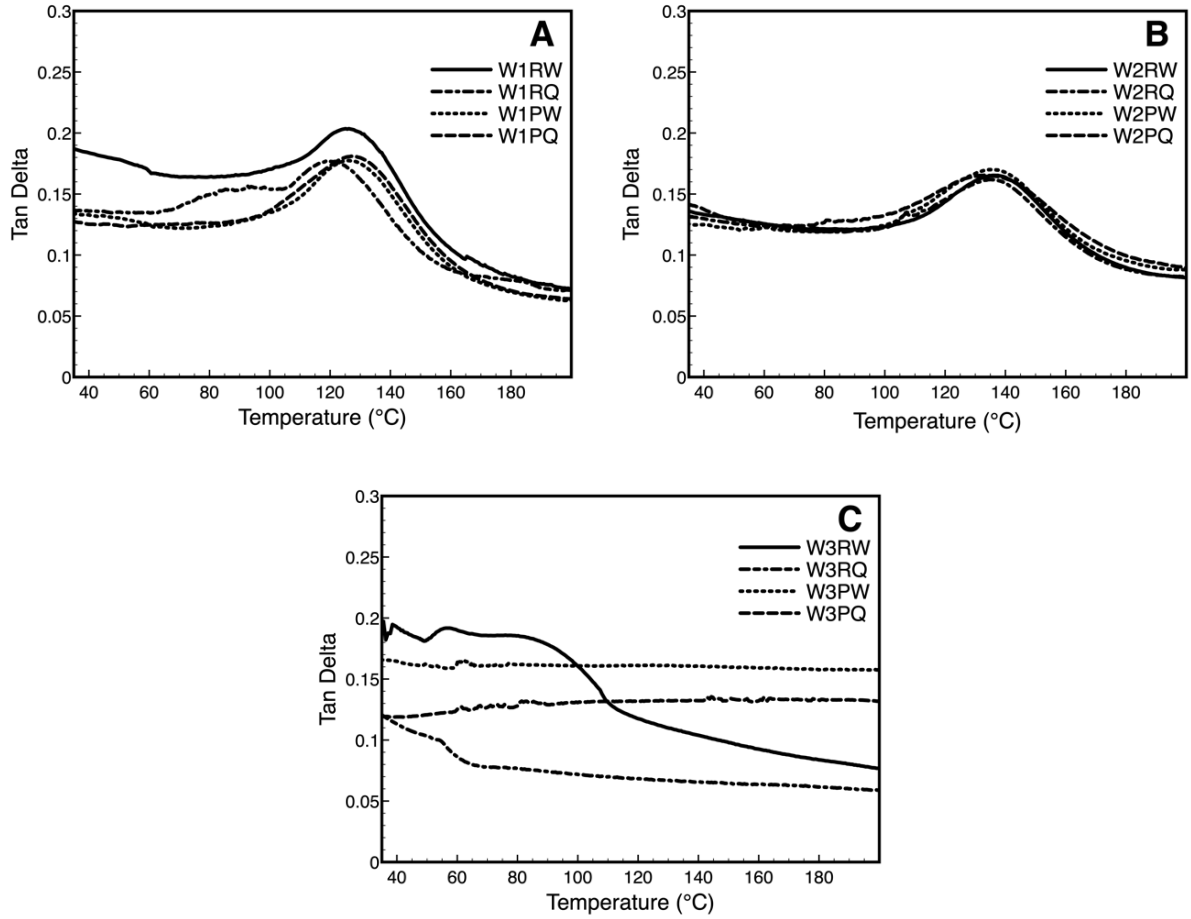


Figure 16. Temperature dependence at 4 Hz of tan delta of W1 (A), W2 (B), W3 (C) of untreated (R) and plasma-treated samples (P) after 7 days of immersion in water (W) and ADBAC (Q).

4.4. Antimicrobial efficacy test

Antimicrobial efficacy tests were carried out in four stages. Firstly, ASTM E2149-13a shaking flask test was applied to evaluate the interaction impact on the antimicrobial performance of the disinfecting wipes directly. Secondly, EN 13727:2012+A2:2015 was employed to evaluate the antimicrobial efficacy of the eluate obtained from the disinfectant-impregnated wipes. Thirdly, EN 16615-2-15 evaluated the overall decontamination efficacy of the disinfecting wipes "in practice". Lastly, the antimicrobial behaviours along storage time were also classified.

4.4.1 Antimicrobial efficacy test with ASTM E2149-13a

In this study, a modified standard ASTM E2149-13a was used. This test method allows direct and complete contact between microorganisms and active ingredients, which eliminates interferences from other parameters (i.e. mechanical action and surface contact). The chosen method gives a straightforward observation of how the disinfectant/substrate interactions impact on the antimicrobial efficacy. The log reduction and standard deviation from the shaking flask test are presented in Figure 17 including both untreated and plasma-treated wipe samples of control and pure ADBAC. ANOVA results (Table S11 in the supporting information) show that the population means of the different testing wipe types are much more significant of the population means of the two bacteria types. W1 showed an excellent log reduction for both Gram-negative and Gram-positive bacteria. Compared to the pure ADBAC untreated sample W1 displayed similar results for *S. aureus* bacteria but lower values for *E. coli*. As proved by XPS analysis, ADBAC in W1 is fully available on the wipe surface, thereby yielding a very good antimicrobial efficacy. After plasma treatment, W1 showed similar results for *E. coli* but an improved bacterial reduction against *S. aureus*. Since the ADBAC efficacy in Gram-positive or Gram-negative bacteria is a function of the N-alkyl chain length, it is reasonably supposed that the plasma generated species interacted with the ADBAC molecule improving the N-alkyl chain orientation in order to have more chains of 12-14 alkyls (optimal for Gram-positive bacteria) then of 14-16 alkyls (optimal for Gram-negative bacteria) [252]. However, it will be necessary to isolate each component of the ADBAC commercial preparation in the function of the N-alkyl chain length and test them separately to confirm this hypothesis. This will be the subject of the following work. Since ADBAC is sold as an unknown mixture of C6H5CH2N(CH3)2RCl, where R can vary from C8H17 to C18H37, it will be necessary to isolate each different quaternary ammonium compound in the ADBAC mixture by chromatography. Then, each compound interacting with the plasma generated species will be analysed by XPS and tested for its antimicrobial performance.

W2 surprisingly exhibited a relatively high log reduction, almost as good as W1 (at least for Gram-positive bacteria), even though strong adsorption of ADBAC was previously observed. The possible reason could be that the function of bacteria reduction to the ADBAC concentration applied is not in a linear relationship, means a small concentration reduction of ADBAC may not cause a dramatic log reduction loss [150]. Besides, reasonable amount of ADBAC can be expected on the surface of W2 due to the polyester composition in W2. The plasma-treated W2 showed a decreased log reduction against *S. aureus* and an increased one in *E. coli*. Also, in this case, the explanation could be the different

interaction kinetic and adsorption of the ADBAC species. It seems that plasma treatment improves the availability of the longer N-alkyl chain ADBAC species (14-16 alkyls), with more affinity for Gram-negative bacteria, on the wipe surface. This can be caused by plasma-induced changes in the critical micelle concentration in which the ADBAC monomers undergo self-assembly to form spherical aggregates showing different adsorption mechanisms (adsorption by electrostatic or hydrophobic interactions) in the function of the wipe material and N-alkyl chains length [253].

W3 cannot be considered as having the antimicrobial effect as a biocide product (Log reduction <3). Both Gram-positive *S. aureus* and Gram-negative *E. coli* bacteria showed significant reductions in antimicrobial efficiency compared to W1 and W2 wipes due to the strong adsorption of ADBAC on cellulose as previously observed. After plasma treatment, a slight improvement in W3's antimicrobial efficacy for Gram-negative *E. coli* can be noted, confirming the ADBAC adsorption reduction with plasma-treated W3 and also validating the hypothesis of selective adsorption of the ADBAC species on cellulose after plasma treatment.

It seems that the antimicrobial test result did not show a significant increase from the plasma-treated wipe samples even though a higher ADBAC concentration was observed from the XPS analysis. However, in the ageing study of the plasma effect on antimicrobial efficacy, it was found that plasma treatment can prolong the release of increased ADBAC on the surface of W1 (polyester) doubling the shelf time (discussion in section 4.4.4). This result, in addition to the fact that only in the pure polyester wipe (W1), it was possible to observe a significant amount of ADBAC on the wipe surface, demonstrated the efficacy of plasma treatment in tightly maintaining the disinfectant for a long time. Besides, plasma treatment is a dry and eco-friendly surface modification method that significantly reduces the consumption of water and the use of chemical stabilizers.

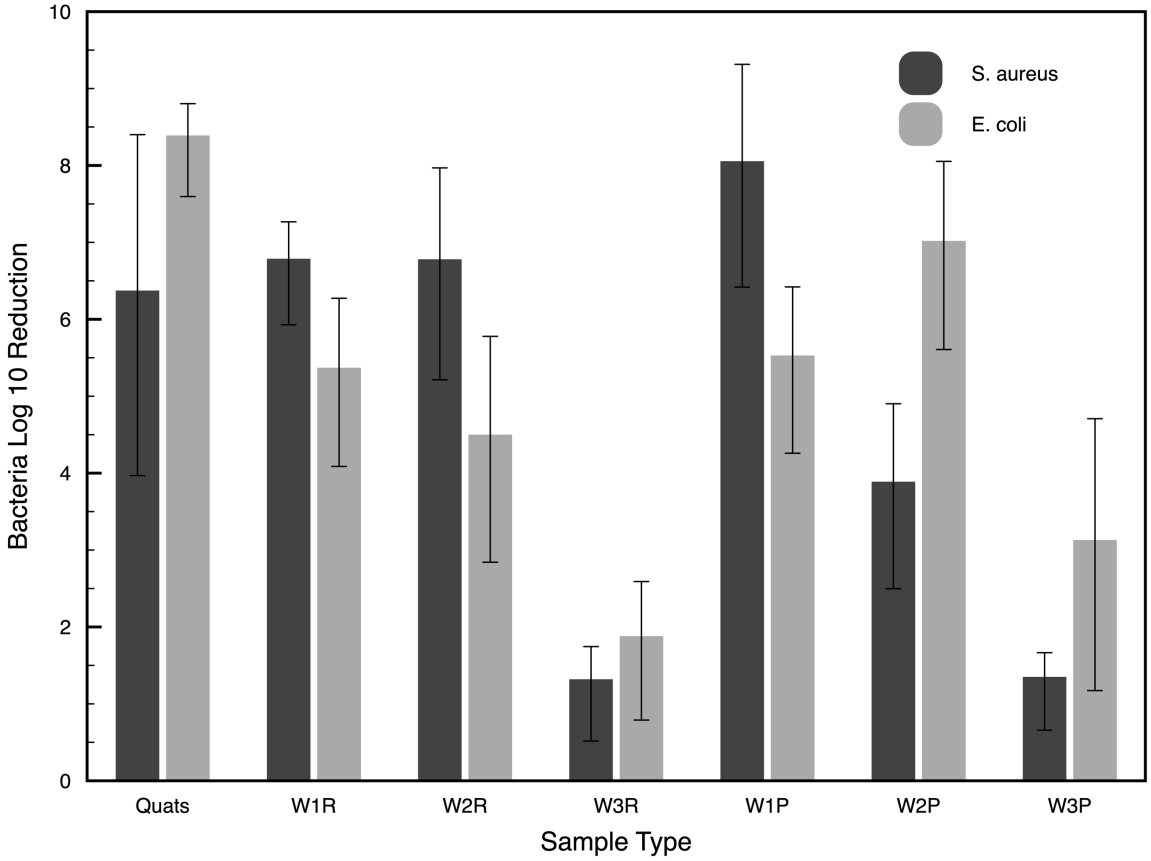


Figure 17. Bacteria (*S. aureus* and *E. coli*) log reduction with the untreated (R) plasma-treated (P) wipe samples in the shaking flask test.

4.4.2 Antimicrobial efficacy test of the eluate with EN 13727:2012+A2:2015

The concentration and concentration reduction of both eluates (E) and remaining bulk (B) liquid were reported in Figure 18 (a – [C] 0.8 g L^{-1} and b – [C] 1.6 g L^{-1}). The ADBAC adsorption is highly dependent on the cellulose content in the wipe samples. In the result from both initial concentrations, the eluate from W3 exhibited the highest concentration reduction, following the eluate of W2 and W1 showed minor concentration reduction. Also, due to the adsorption of ADBAC on cellulose material, there was surprisingly a significant difference between the concentration of the bulk and eluate in Wipe 3 sample. The concentration of the eluate was much lower than the one remaining in the bulk solution. In theory, the textile substrate works as a carrier transferring the disinfectant solution from the stock onto the target surface. When the adsorption of active ingredients on the textile substrate takes place, with sufficient immersion time, the adsorption reaches an equilibrium. An equal solution concentration is expected in both the bulk solution and solution carried by the textile substrate. However, it seems

that the adsorbed ADBAC was tightly bonded to the wipe and cannot be released in the liquid on the surface after compression (the wringing process) of the textile substrate. This property resulted in a lower concentration in the eluate solution than in the remaining bulk. This outcome should alert people about the real quantity of the disinfectant applied for surface disinfection when adsorption of active ingredients from disinfectant onto textile substrate takes place. Even if there is a sufficient amount of disinfectant remains in the bulk solution, the final concentration of the disinfectant used on the target surface may be lower. This finding confirms again there are very limited active ingredients (more than the expected value measured from the remaining bulk solution) that eventually works for disinfection purpose. Comparing the results from the two initial concentrations, there seems to be no influence from the initial concentration on the behaviour of ADBAC adsorption (with liquor ratio 3.5g/20mL and immersion time 20 hours). However, the difference between the concentrations of eluates and the bulk solution is more pronounced with higher initial concentration (0.2 g L^{-1} for initial concentration 1.6 g L^{-1} and 0.14 g L^{-1} for initial concentration 0.8 g L^{-1}) in the case of sample W3.

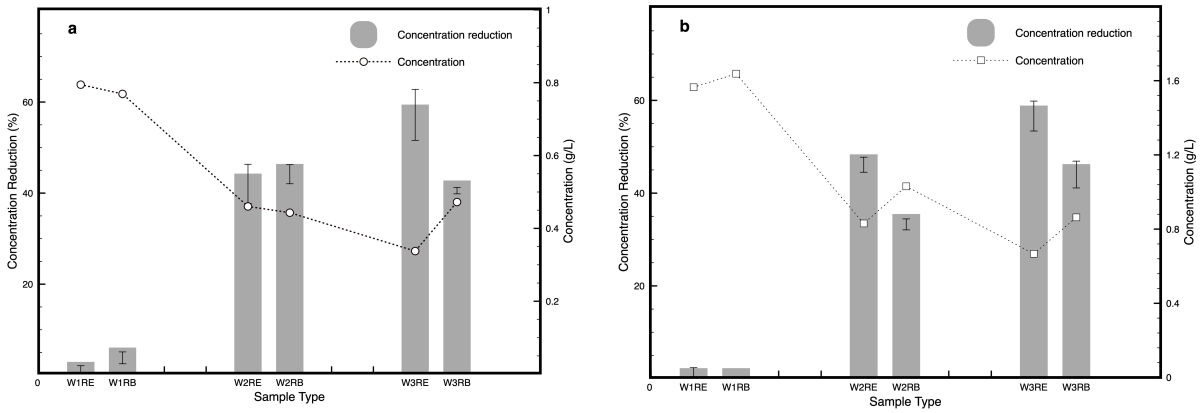


Figure 18. The concentration and concentration reduction of both eluates (E) and remaining bulk (B) liquid (a – [C] 0.8 g L^{-1} and b – [C] 1.6 g L^{-1}) when ADBAC solution encountered with untreated wipe samples.

Both untreated and plasma-treated wipe samples were tested against *S. aureus* (Figure 19), There was no log reduction observed from the water control of untreated and plasma-treated wipe samples. Moreover, there was no significant difference between the water control of different wipe samples in terms of bacteria log reduction. This gave an equal baseline to all three types of wipes when compare the result from the ones immersed in ADBAC solution.

Within their standard deviation, the eluate from control W1 showed its log reduction as good as the pure ADBAC solution. No *S. aureus* survivals were observed from their plate counting with 0 dilution factor. With ADBAC concentration of 0.8 g L^{-1}, the negligible QACs adsorption from W1 has no impact on the result of log reduction. However, plasma-treated W1 demonstrated a decrease in log reduction result. The plasma-treated W1 tends to obtain a higher ADBAC adsorption in all liquor ratios investigated in the absorption and adsorption test, this explained the decreased log reduction from plasma-treated W1 eluate. Surprisingly, EN 13727 is sensitive enough to detect such a small change in the concentration decrease. Due to the concentration reduction in the eluate in the control W2 (Figure 19), the log reduction is correspondingly reduced to less than 4. But the plasma-treated W2 gives an abnormally increased log reduction compared with the control. In the investigation of absorption and adsorption test, plasma-treated W2 used to have a higher concentration reduction, that expected to lead to a lower log reduction against *S. aureus* in the EN 1372 test. However, plasma effect on the polyester surface of W2 and its interactions with the disinfectant seem to contribute to some extent to the antimicrobial properties in the W2 eluate. W3 completely lost its bactericidal ability (Figure 19). Regarding the plasma-treated W3, a slight increase in log reduction result can be observed, yet it is still below the bactericidal requirement from the standard. It showed the limitation of plasma treatment in reducing the binding of QACs onto cellulose substrate.

In the test against *P. aeruginosa*, due to the weak bactericidal ability of QACs against Gram-negative bacteria, the concentration of QACs for the immersion rose up to 1.6 g L^{-1}. Nevertheless, biofilm from *P. aeruginosa* was observed in the plate counting. The result showed poor bactericidal performance from the eluates of wipes. Compare with the pure ADBAC solution, *P. aeruginosa* log reduction from the eluates of W1 was nearly half reduced. For eluate of W2, the log reduction dropped to below 2 and eluate from W3 shows no bactericidal action at all, the same result as its water control. The ADBAC performed very poorly bactericidal against *P. aeruginosa*. However, taking the concentration result from UV – spectrophotometer into account, it seems that a minor ADBAC adsorption can make a significant difference in the log reduction against *P. aeruginosa* (comparing the result from pure ADBAC and eluate of W1). It implied the eluate from the wipe sample was somehow (with possible addition of free fibrils in the eluate) compounding the ability of biofilm growing of *P. aeruginosa*.

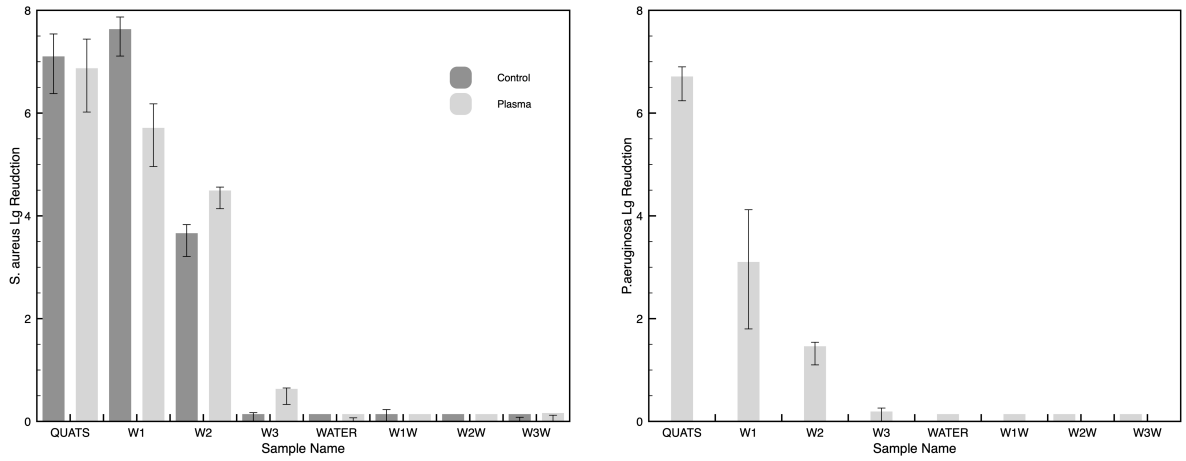


Figure 19. Log reduction against *S. aureus* and *P. aeruginosa* of the eluates.

4.4.3 Antimicrobial efficacy evaluation in practice (EN16615-2015)

Comparing the log reduction from field 1 (Na) of three types of wipe samples, W1 was able to kill all the bacteria (W1Na); W2 was able to eliminate all the bacteria except in 1 repetition with only few CFUs left on the plate (W2Na); on the contrary, W3 did not obtain any bactericidal ability (Figure 20). The performance of W3 is as worse as its water control. Within its standard deviation, it can be interpreted as W3 soaked in ADBAC is equal to W3 in water in terms of its antimicrobial efficacy, which means there is only the removal of bacteria from the wiping action instead of the bactericidal effect, which is a high risk of pathogen transmission during the wiping action. Moreover, the bacteria log accumulation result on fields 2-4 (WS) of the water control in all wipe samples indicated that the wipe sample without any lethal action on the bacteria can boost/enhance the spreading of bacteria in the other wiping area. Different water control log reductions were reported in Figure 20 shows that different materials possess different bacterial removal abilities [54]. Besides, the test result from W1 and W2 (NaS) exhibits less risk of bacteria spreading to other wiping area since most bacteria on site were killed with sufficient ADBAC solution released on field 1. However, the test result from W3 demonstrated great risk of bacteria spreading, just as in its water control. The testing result indicates, even though the wipe itself has the ability to remove the bacteria, the risk of bacteria spreading is very high with only detergent soaked wipes.

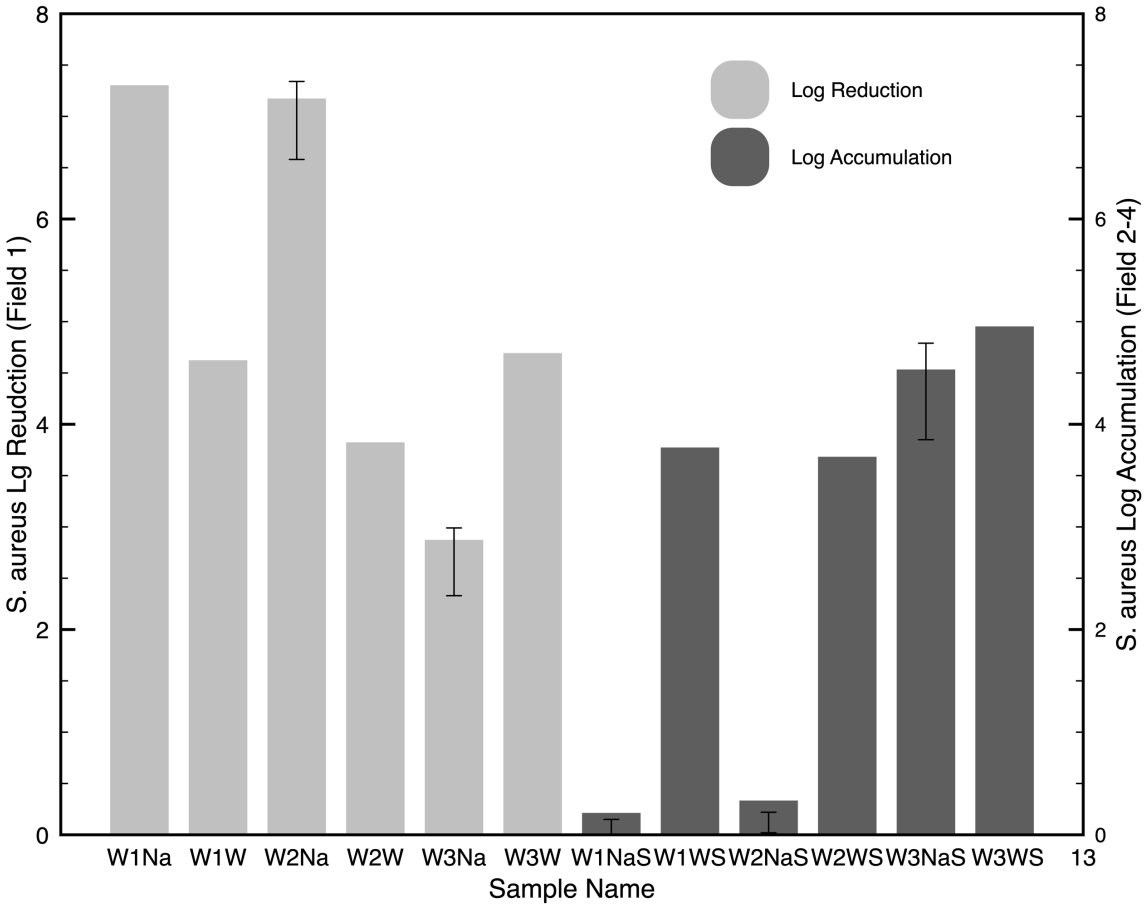


Figure 20. EN 16615 test against *S. aureus* of ADBAC immersed wipe samples (Na) and their water control, test result displayed Na/W - log reduction of Field 1, NaS/WS - bacteria accumulation from Field 2-4.

4.4.4 Antimicrobial efficacy changing over storage time

How the interaction impacts on the antimicrobial efficacy along time was studied with test method ASTM E2149-13a The antimicrobial efficacy as Log reductions were presented in Figure 21 in the function of the storage time (30 min – 30 days) and bacterial strains (Gram-positive and Gram-negative). The reason choosing this test method was explained elsewhere in the thesis. ANOVA analysis showed that storage time did not have any significant influence on the antimicrobial efficacy of the control and plasma-treated wipe samples (Table S12 and Table S13 in the supporting information). However, sample type has a significant influence on the Log reduction (control wipe with p-value 6.30615E-05 and plasma-treated wipe sample with p-value 2.55926E-05). Due to the adsorption of ADBAC active ingredient on cellulose material, untreated and plasma-treated W3 showed the lowest Log

reduction among all the testing samples. The Log reduction result corresponds to the previously observed quaternary ammonium salt inactivation in cotton due to the adsorption on the fibres that cannot release the antimicrobial agents [46, 48]. The adsorbed active ingredients lost its bactericidal function (Log reduction <3) and thereby failed the antimicrobial efficacy test. In the case of the other wipe samples, W1 and W2, the wipes were acting like a carrier that transfer the ADBAC solution from the bulk onto the target surface. Thus, the Log reduction showed almost the same result as the pure ADBAC solution. In addition, ADBAC was clearly more effective in Gram-negative *E. coli* than Gram-positive *S. aureus* for every type of wipe including plasma-treated ones. ANOVA analysis of untreated W1 and W2 showed that the storage time has a significant influence on their Log reduction of *E. coli* (control wipe samples with a p-value of 0,036 and plasma-treated wipe samples with a p-value of 0,005).

Despite the great variability of the antimicrobial performance, plasma treatment displayed a significant effect in W1 and W2 wipes against *S. aureus* and for W1 against *E. coli.,* Plasma-treated W1 wipe enhance the antimicrobial efficacy against *S. aureus* increasing the Log reduction from 2.5 to 4.5 at day 1 and from 6 to 8 after 15 days while after 30 days both untreated and plasma-treated samples showed a significant reduction in antimicrobial efficacy. Similar behaviour can be observed in W2 wipe but only up to 7 days. In the case of *E. coli,* the plasma treatment seems to preserve the antimicrobial activity during storage time for W1 wipe while no changes in W2 can be depicted. As expected, plasma treatment did not affect the very low antimicrobial activity in cotton-based W3 wipe. Plasma treatment in polyester wipes (W1) was able to minimize the main drawback on ADBAC absorption, namely the hydrophobicity of the polyester surface which did not allow ADBAC to remain in the wipe. Plasma treatment can improve the surface adsorption of ADBAC due to the plasma-generated oxygen species that allow a controlled release of the disinfectant over storage time conditions [254].

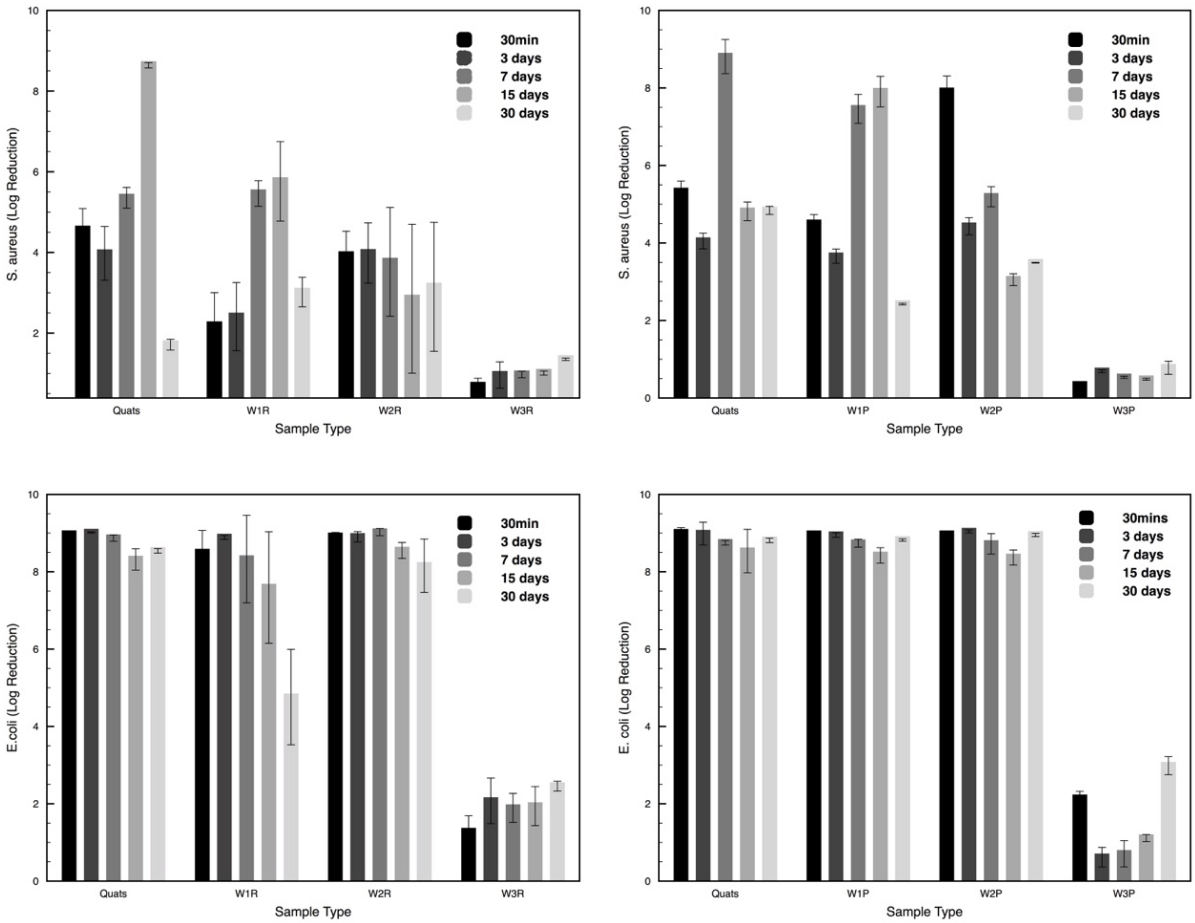


Figure 21. Log reduction of *S. aureus* and *E. coli* on the untreated (R) and plasma-treated (P) disinfecting wipes stored for 30 min, 3, 7, 15, and 30 days.

Chapter 5

Conclusion and Outlook

5. CONCLUSION AND OUTLOOK

5.1. Conclusion

The use of pre-impregnated disinfecting wipes is one of the most efficient and prevalent methods for the decontamination of high-touch environmental surfaces and non-critical medical devices in hospitals and other healthcare centres. There is evidence to support the importance of disinfecting wipes in preventing cross-contamination and spread of HCAIs. Nowadays, the most reliable method that can be used in hospitals seems to be the one using ready-to-use disinfecting wipes because of its lower disinfection failure risk. There are several variables including the internal and external factors that influence the effectiveness of disinfecting wipes in the decontamination process. The interaction between disinfectant and textile substrate becomes the biggest encumbrance for its disinfection performance and barrier of confident use in hospitals. The following issues could have an impact on the interaction.

- Material compatibility (combination of wipe and disinfectant)
- Liquor ratio (wipe mass/disinfection solution volume)
- Contact time (of disinfectant and wipes)
- Storage time

The PhD project was therefore developed to understand the interaction mechanism between QACs and textile wipes incorporated with DBD plasma treatment, as well as their performance during ageing.
W1 (100% polyester) showed the best disinfectant performances and roughly no adsorption of ADBAC. W2 (55% cellulose and 45% polyester) revealed a medium response in adsorption of ADBAC, but similar antimicrobial effectiveness to W1, whereas the plasma-treated ones displayed an increased adsorption effect due to the hydrophilic nature of the plasma-generated species on the polyester component. However, the antimicrobial performance of plasma-treated W2 showed an opposite behaviour compared to the untreated sample because of the different adsorption mechanisms of the disinfectant that depending to the type of wipe material or N-alkyl chains length of ADBAC which adsorption can be ruled by electrostatic or hydrophobic interactions. W3 (100% cellulosic material) achieved the major adsorption of ADBAC resulting in the highest values of concentration reduction (C_R). Despite plasma

treatment significantly reduced the ADBAC adsorption of about 50%, both untreated and plasma-treated samples showed the lowest antimicrobial efficacy due to the high adsorption and negative interaction of ADBAC with the OH groups in cellulose. It is important to note that liquor ratio (fabric mass in gram/liquid volume in mL) and immersion time in the first 30 minutes, has a relatively substantial impact on the investigated variables. A small liquor ratio can yield to low C_R, high C_Q and R_w leading to the higher available disinfectant active ingredient in the wipe.

X-ray photoelectron spectroscopy (XPS) analysis on the control wipe samples showed that the absorbed ADBAC was more present on the surface of W1 comparing with the other wipe samples. XPS analysis result also demonstrated the incorporation of reactive oxygen species on the fibre surface of plasma-treated polyester-containing wipes, resulting in higher adsorption of ADBAC on the surface. Laser scanning microscopy demonstrated the plasma etching effect in smoothing the surface of the cotton wipe reducing the adsorption of ADBAC.

In the characterization of its ageing performance, plasma treatment showed to have a significant effect on the thermo-mechanical properties of the wipes slightly reducing the force at break and elongation in water and ADBAC treated wipes (W1 and W2). Plasma-treated W3 cotton wipe did not show any loss but a slight increase in elasticity during storage time in weft direction due to the reducing of the cellulose fibre swelling. DMA analysis demonstrated that the blend wipe (W2) was not affected in its viscoelastic properties and highlighted the opposite mechanical behaviour comparing with W1 and W3 wipes. The presence of plasma treatment in W1 improved the elastic response of the wipe limiting the mobility of the polymeric chains of polyester, while the plasma treatment in W3 clearly increased the non-elastic strain component due to the etching effect.

Regarding the antimicrobial performance along the storage time, DBD plasma treatment was able to duplicate the shelf life in term of antimicrobial efficacy of pure polyester wipes (W1) up to 15 days for Gram-positive bacteria and 30 days for Gram-negative bacteria compared to the untreated samples. In a less extend, also in the blend polyester/cotton wipe (W2) plasma treatment was able to enhance the antimicrobial efficacy of about 30% for Gram-positive bacteria and continue to have excellent activity in Gram-negative bacteria. The adsorption of ADBAC on cellulose (W3) completely blocked the biocidal effect of active ingredients, which is a high risk for infection control.

Overall, on one hand, this work confirms once again the negative impact of cellulose in the efficacy of QACs for disinfection. On the other hand, plasma treatment applied to polyester-containing wipes reveals to be an effective way to improve QACs concentration on the wipe surface for improved

antimicrobial efficacy, and duplicate shelf time by slowly releasing adsorbed QACs. Moreover, this method allows the use of pure polyester as effective wiping material for surface disinfection eliminating the major drawback of pure polyester, its high hydrophobicity. This work opens the way for a new class of wiping materials with improved antimicrobial efficacy using a low-cost and environmental-friendly plasma technique. The outcome research knowledge is important to ensure hospitals daily workflow from unnecessary risk of infection outbreak and to complement the products' user manual of disinfectant and wipes in the market.

5.2. Outlook

Based on the literature review, standards to date remain some drawbacks in testing the effectiveness of DIWs. More realistic condition simulation and differentiating between the mechanical removal of inoculum from a surface and chemical inactivation of the test microbe are called for attention. Divergent outcomes with different test standards can be suspected. A guideline for comparable results between various test standards is in demand. It is important to note that disinfecting wipes decontamination efficacy testing standards is the validation step before the disinfectant-impregnated wipe products launched into the market and further used in hospitals.

From this PhD, it is noticed that the plasma-treated wipe changed the adsorption mechanisms (adsorption by electrostatic or hydrophobic interactions) in the function of the wipe material and N-alkyl chains length. Future research direction can study more in detail about the adsorption behaviour change due to the plasma treatment. Other advanced surface modification technology, such as polymer-functionalization, could also be considered for improving the disinfection efficacy of disinfectant-impregnated wipes. Also the ability of disinfectant-impregnated wipes against biofilm is also in urgent need for future research due to the ever-increasing evidences of biofilm in the presence of HCAIs.

Since a good cleaning and disinfection protocol is essential for hospital infection prevention and control, the development of more environmentally sustained products and processes, avoiding resources wasting is always required. Considering the waste management of disposable wipes, biodegradable wipes with sufficient antimicrobial efficacy are in need for the future market.

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ANNEX

(Supporting Information)

ANNEX I – Pre-selection of wipe samples

Table S1. Areal density of wipe samples (g m^{-2}), data represented 5 repetitions, their average (Avg.) and ± SD/CV%.

Sample Name	1	2	3	4	5	Avg.	SD	CV%
1.PET	40.80	40.60	40.50	40.50	42.20	**40.92**	0.65	1.59
2.PET (woven)	101.30	101.60	101.30	100.60	101.90	**101.34**	0.43	0.43
3.PA (woven)	110.10	110.60	110.30	110.90	110.40	**110.46**	0.27	0.25
4.CEL/PP	90.60	91.00	90.40	92.60	91.60	**91.24**	0.79	0.87
5.CEL/PET	53.50	53.30	54.90	53.70	54.40	**53.96**	0.60	1.11
6. CEL/PET	68.90	69.80	70.70	68.90	69.60	**69.58**	0.67	0.96
7.Woodpulp/PET	59.10	62.90	61.80	61.90	61.60	**61.46**	1.26	2.05
8. Cotton (woven)	118.30	118.84	118.17	118.73	119.54	**118.72**	0.48	0.41

The measurement was performed under standard condition of 20 ± 2°C and 65 ± 2% RH. All the wipe samples were conditioned for 48 hours before testing. The average areal density of all the chosen wipe samples were reported in Table S1. The measurement values are between 40g m^{-2} and 120g m^{-2}. The wipe samples used in this project belong to the category disposable and semi-disposable wiping cloths.

Table S2. Fabric thickness measurement of wipe samples (mm), data represented 10 repetitions, their average (Avg.) and ± SD/CV%.

Sample Name	1	2	3	4	5	6	7	8	9	10	Avg.	SD	CV%
1.PET	0.37	0.35	0.34	0.34	0.34	0.39	0.39	0.33	0.38	0.35	**0.36**	0.02	5.93
2.PET (woven)	0.40	0.45	0.42	0.41	0.44	0.46	0.43	0.45	0.41	0.47	**0.44**	0.02	5.15
3.PA (woven)	0.61	0.59	0.62	0.58	0.60	0.56	0.59	0.58	0.57	0.57	**0.59**	0.02	3.04
4.CEL/PP	0.99	0.96	0.93	0.98	0.93	0.96	0.92	0.94	0.96	1.01	**0.96**	0.03	2.86
5.CEL/PET	0.45	0.49	0.43	0.46	0.45	0.43	0.49	0.44	0.46	0.47	**0.46**	0.02	4.47
6. CEL/PET	0.54	0.54	0.56	0.55	0.56	0.56	0.54	0.52	0.53	0.51	**0.54**	0.02	3.02
7.Woodpulp/PET	0.58	0.60	0.61	0.60	0.59	0.62	0.61	0.61	0.60	0.64	**0.61**	0.02	2.57
8. Cotton (woven)	1.06	0.99	1.02	0.93	0.88	0.94	0.99	1.03	0.97	0.99	**0.98**	0.05	5.09

Fabric thickness is defined as perpendicular distance through the fabric, which determines the dimension between the upper and lower side of the fabric. (Kremenakova, D., Kolcavova Sirkova, B., Mertova, I.: Internal standards, Research centre, Liberec 2004) The thickness of the fabrics was measured according to the standard ASTM D1777-96 (2015) with the digital thickness gauge M034 A at a pressure of 100 Pascal. The test was performed under standard condition of 20 ± 2°C and 65 ± 2% RH. All the wipe samples were conditioned for 48 hours before testing. Every sample took 10 repetitions of measurement. The average and standard deviation were calculated and reported in Table S2. All the wipe samples were design for disposable use, therefore, the thickness of the wipe samples are relatively small below 1 mm. Sample 4.CEL/PP and 8. Cotton presented the highest values of thickness among all the measurements, 0.96mm and 0.98mm respectively. Contrarily, sample 1.PET has the smallest thickness value of 0.36mm. In general, sample thickness depends on the material composition and fabric construction technique.

Table S3. Air permeability of wipe samples (200pa), data represented 10 repetitions, their average (Avg.) and ± SD/CV%.

Sample Name	1	2	3	4	5	6	7	8	9	10	Avg.	SD	CV%
1.PET	2950	2960	2950	2900	2860	2990	2910	2920	2860	2940	**2924**	40	1
2.PET (woven)	93.6	88.7	87.0	86.5	87.2	87.1	88.0	90.0	89.0	89.7	**88.7**	2	2
3.PA (woven)	63.8	62.1	60.7	60.9	58.4	56.1	55.3	55.2	56.3	55.7	**58.4**	3	5
4.CEL/PP	614	597	627	616	625	612	596	616	581	626	**611**	14	2
5.CEL/PET	803	816	794	825	799	832	795	825	829	818	**814**	14	2
6. CEL/PET	645	639	626	602	652	614	632	610	629	647	**630**	17	3
7.Woodpulp/PET	2250	2330	2290	2350	2320	2260	2290	2360	2240	2260	**2295**	41	2
8. Cotton (woven)	917	948	950	938	943	921	913	898	901	886	**922**	21	2

Air permeability of wipe samples was carried out according to standard ASTM D737-04 (2008) with a head area of 20cm^2 in differential pressure of 200pa. The rate of air flow passing perpendicularly through a known area of fabric is adjusted to obtain a prescribed air pressure differential between the two fabric surfaces. From this rate of air flow, the air permeability of the fabric is determined. Air permeability was measured on an FX 3300 air permeability tester by Textest AG, Switzerland, at the standard condition of 20 ± 2°C and 65 ± 2% RH. All the wipe samples were conditioned for 48 hours before testing. 10 measurement repetitions were made for every wipe sample. (In case of the CV of the 10 measurements is more than 10%, more repetitions were performed until achieve the CV within 10%). The wipe samples exhibited completely different performance in air permeability measurement. Among the result, sample 1. PET showed a highest value of 2907 L m^{-2} s^{-1} in the air permeability measurement, whereas sample 3. PA showed the lowest value of 88.68 L m^{-2} s^{-1}. Generally, the investigation of air permeability property of textiles was carried out separately in woven and nonwoven fabrics. For woven fabric, the voids between the weft and warp yarns plays a major role in the air permeability performance. In addition, the air permeability of the woven fabric is affected by several factors: fabric structure, the warp and weft densities, the twist in yarns, the size of the yarn and the yarn structure [255]. In the case of nonwoven fabric, research has discovered that the air permeability of the fabric is influenced by bonding method (needling, stitch-bonding, adhesive-bonding etc.) needling/stitch density

(binder content), fabric thickness, fabric weight, fabric density, and fibre diameter. Particularly the areal density of the fabric is found to be most closely related to the air permeability and that the air permeability is almost directly proportional to the reciprocal of the weight per unit area, namely the areal density [256]. In this project, wipe sample 2. PET, 3. PA and 8. Cotton are woven fabrics and the rest wipe samples are nonwoven structured. Due to the structure difference, the air permeability of woven wipe sample 2 and 3 are significantly smaller than the other nonwoven wipe samples. Sample 8. Cotton is a woven structured fabric made from 100% cotton fibre. Studies has showed yarn hairiness has a great influence on the air permeability behaviour due to the change of the inter-yarn pore size. (Havlová, M. (2013). Air permeability and constructional parameters of woven fabrics. Fibres & Textiles in Eastern Europe.) Yet the hairiness of natural cotton fibre yarn and synthetic filaments are completely different which leads to quite different air permeability values. It is interesting to notice that the measurement of air permeability in this project confirmed the research conclusion from V. K. Kothari et al. [256] excluding sample 7.Woodpulp/PET and 8.Cotton. The air permeability values grew with the decline of the areal density values in the wipe samples. In the data of areal density (Table S1), the values increased with the order sample 3. PA > 2. PET woven > 4. CEL/PP > 6. CEL/PET > 5. CEL/PET > 1. PET. Whereas, the measurement of air permeability values exhibited in the order of 3. PA < 2. PET woven < 4. CEL/PP < 5. CEL/PET < 6. CEL/PET <1. PET. The reasons for the exceptional performance of sample7. Woodpulp/PET and 8. Cotton can be explained as following: Sample 7. Woodpulp/PET is a composite nonwoven wipe made of woodpulp in the core layer and laminated with polypropylene on the surface, demonstrating a different air permeability behaviour. The hairiness of cotton yarn can be explained as the reason for its particular air permeability value.

Table S4. Coefficient of friction of wipe samples by Frictorq, data represented 5 repetitions, their average (Avg.) and ± SD/CV%.

Sample Name	Front/Back	1	2	3	4	5	Avg.	SD	CV%
1. PET	F	0.1731	0.1729	0.1707	0.1746	0.1678	**0.1718**	0.003	1.54
	B	0.1664	0.1629	0.1635	0.1675	0.1525	**0.1626**	0.006	3.66
2. PET (woven)	F	0.1633	0.1336	0.1592	0.1582	0.1375	**0.1504**	0.014	9.13
	B	0.1357	0.1544	0.1354	0.1400	0.1609	**0.1453**	0.012	8.03
3. PA (woven)	F	0.1617	0.1691	0.1659	0.1712	0.1681	**0.1672**	0.004	2.16
	B	0.1677	0.1711	0.1706	0.1725	0.1728	**0.1709**	0.002	1.19
4. CEL/PP	F	0.1813	0.1898	0.1906	0.1922	0.1983	**0.1904**	0.006	3.20
	B	0.1747	0.1884	0.1827	0.1828	0.1784	**0.1814**	0.005	2.85
5. CEL/PET	F	0.1727	0.1723	0.1744	0.1762	0.1753	**0.1742**	0.002	0.96
	B	0.1568	0.1601	0.1559	0.1589	0.1597	**0.1583**	0.002	1.16
6. CEL/PET	F	0.1746	0.1780	0.1701	0.1732	0.1767	**0.1745**	0.003	1.77
	B	0.1572	0.1577	0.1526	0.1528	0.1583	**0.1557**	0.003	1.79
7. Woodpulp/PET	F	0.1788	0.1704	0.1767	0.1743	0.1739	**0.1748**	0.003	1.81
	B	0.1594	0.1589	0.1523	0.1625	0.1598	**0.1586**	0.004	2.38
8. Cotton (woven)	F	0.1856	0.1927	0.1969	0.2042	0.1875	**0.1934**	0.008	3.88
	B	0.1943	0.1906	0.1982	0.198	0.1813	**0.1925**	0.007	3.63

Table S5. Average Coefficient of friction of wipe samples by Frictorq and ± SD/CV%.

Sample Name	Front	SD	CV%	Back	SD	CV%
1. PET	0.1718	0.0026	1.54	0.1626	0.0059	3.66
2. PET (woven)	0.1504	0.0137	9.13	0.1453	0.0117	8.03
3. PA (woven)	0.1672	0.0036	2.16	0.1709	0.0020	1.19
4. CEL/PP	0.1904	0.0061	3.20	0.1814	0.0052	2.85
5. CEL/PET	0.1742	0.0017	0.96	0.1583	0.0018	1.16
6. CEL/PET	0.1745	0.0031	1.77	0.1557	0.0028	1.79
7. Woodpulp/PET	0.1748	0.0032	1.81	0.1586	0.0038	2.38
8. Cotton (woven)	0.1934	0.0075	3.88	0.1925	0.007	3.63

Coefficient of friction of the wipe samples was measured by FRICTORQ device developed by University of Minho, Portugal under the standard condition of 20 ± 2°C and 65 ± 2% RH. All the wipe samples were conditioned for 48 hours before testing. FRICTORQ characterises the friction coefficient between tow contacting surfaces by measuring the dragging torque. The principle is based on an annular shaped flat upper body (with attachment of a precision reaction torque sensor) rubbing against a lower flat surface (where the textile material can be placed). The lower surface which contains the textile material rotates around a vertical axis at a constant angular velocity. The kinetic friction coefficient is then proportional to the level of the dragging torque measured by a precision reaction torque sensor [257]. The measurement of every wipe samples was replicated five times. From the T test of all the seven samples in the experiment, all the p-values were below 0.05, indicating that the difference between the two groups (Front and Back) is statistically significant (5% level of significance). The kinetic (dynamic) coefficient of friction of all the wipe samples are not found to differ greatly. Though wipe sample 2. PET (woven) showed a lower kinetic coefficient of friction than all the other wipe samples and by contrary, wipe sample 8. Cotton presented a much higher kinetic coefficient of friction in both front side and back side measurements. The front side kinetic coefficient of friction value of sample 4 CEL/PP are slightly smaller than wipe sample 8. Cotton. The values of front side of the wipe sample were in an order of 8. Cotton > 4. CEL/PP > 7. CEL/PET > 6. CEL/PET > 5. CEL/PET > 1. PET > 3. PA (woven) > 2. PET

(woven). The order of value level from the measurement of the back side of the wipe samples was as following: 8. Cotton > 4. CEL/PP > 3. PA (woven) > 1. PET > 7. CEL/PET > 5. CEL/PET > 6. CEL/PET The values of sample 5, 6 and 7 in either side exhibited no significant difference since they possess the similar content of cellulose and polyester.

Table S6. Vertical wicking test values of wipe samples in Machine (M) and Cross (C) direction (or Warp/Wa and Weft/We for woven fabric).

Sample Name	Direction	1min	2min	3min	4min	5min	6min	7min	8min	9min	10min
1.PET	**MD**	0.07	0.08	0.11	0.12	0.13	0.14	0.15	0.16	0.17	0.17
	CD	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.02	0.02	0.02
2.PET (woven)	**Wa**	8.63	10.07	11.10	11.87	12.60	13.17	13.70	14.23	14.73	15.23
	We	8.90	10.35	11.55	12.45	13.25	13.95	14.63	15.13	15.53	15.88
3.PA (woven)	**Wa**	4.90	5.40	5.80	6.20	6.50	6.93	7.10	7.67	7.93	8.13
	We	4.97	5.87	6.63	7.10	7.67	8.23	8.67	9.10	9.47	9.83
4.CEL/PP	**MD**	7.97	8.47	8.83	9.00	9.13	9.30	9.30	9.40	9.47	9.57
	CD	7.97	8.47	8.83	9.00	9.13	9.30	9.30	9.40	9.47	9.57
5.CEL/PET	**MD**	7.53	8.53	9.03	9.53	9.97	10.27	10.50	10.87	11.17	11.47
	CD	6.40	7.37	7.97	8.27	8.67	8.90	9.17	9.33	9.47	9.60
6.CEL/PET	**MD**	7.35	8.55	9.33	10.03	10.63	11.18	11.83	12.25	12.50	12.73
	CD	5.77	6.77	7.30	7.80	8.30	8.70	8.97	9.30	9.47	9.67
7.Woodpulp/	**M**	5.63	6.20	6.50	6.63	6.87	7.10	7.23	7.43	7.57	7.67
PET	**CD**	4.33	4.83	5.17	5.37	5.57	5.77	5.97	6.03	6.13	6.27
8.Cotton(woven)	**Wa**	6.07	7.07	7.83	8.37	8.83	9.30	9.73	9.97	10.23	10.53
	We	5.80	6.80	7.30	7.80	8.20	8.63	8.83	9.20	9.47	9.73

Transport of water through textiles, namely the wicking properties of textiles, takes place as the phenomenon of capillarity [258]. Capillarity is the ability of liquids to penetrate fine pores and cracks with wettable walls and be displaced from those with non-wettable walls [259]. The capillary action, is governed by the properties of the liquid, liquid-medium (in this case, textile fabric) surface interactions, and geometric configurations of the pore structure in the medium [260]. By the wicking process, the fabric displaces fibre-air interface with a fibre-liquid interface [261]. Vertical wicking test were conducted under the standard atmosphere of 20 ± 2°C and 65 ± 2% RH. All the wipe samples were conditioned for 48 hours before testing. The wipe samples were cut in size of 20cm x 2.5 cm along the machine

(warp-wise in woven fabric) and cross (weft-wise in woven fabric) direction. The prepared wipe samples were suspended vertically with their bottom end dipped in a reservoir of distilled water with blue dye (for distinct reading of the measurement). To make sure that the bottom end of the wipe sample could be immersed vertically at a depth of 30mm into the water, a 1.2g clip was used for the bottom end of each wipe sample. The wicking heights, the distance travelled by water on vertical strip, were measured every minute up to 10 mins. three replicates were carried out for every wipe sample.

Table S7. Horizontal wicking measurement of wipe samples (g).

	1min	2min	3min	4min	5min	6min	7min	8min	9min	10min
1.PET	1.12	2.30	3.41	4.36	5.19	5.94	6.64	7.31	7.94	8.54
2.PET (woven)	4.57	8.06	10.08	11.75	12.98	13.96	14.75	15.36	15.89	16.43
3.PA (woven)	2.81	5.02	6.85	8.37	9.71	10.83	11.78	12.58	13.26	13.81
4.CEL/PP	2.44	8.18	6.97	8.70	10.36	11.73	13.09	14.21	15.13	16.04
5.CEL/PET	1.85	3.16	4.33	5.38	6.29	7.13	7.86	8.50	9.08	9.57
6.CEL/PET	3.13	5.02	6.55	7.85	8.98	9.97	10.81	11.52	12.17	12.79
7.Woodpulp/PET	2.76	4.40	5.86	7.15	8.23	9.16	9.98	10.74	11.42	11.93
8.Cotton (woven)	7.31	10.72	13.27	14.96	15.80	16.65	17.37	18.06	18.65	19.23

Horizontal wicking is the transmission of water through the thickness of a fabric, i.e. a single drop wicking into a fabric [261]. Horizontal wicking test were conducted under the standard atmosphere of 20 ± 2°C and 65 ± 2% RH. All the wipe sample were conditioned for 48 hours before testing. The wipe samples were cut in the size of 20cm x 20cm and placed horizontally between two glass plates with a tiny drop of water placed on the fabric. The water absorption took place by wicking and wetting through the pores. The water was supplied continuously from a reservoir with 80 g of water by siphoning to the bottom of the specimen. The reservoir was kept on an electronic balance, which enables the recording of the water mass absorbed by the fabric. The wicking was measured every minute up to 10 mins and expressed as the weight of absorbed water. three replicated were carried out for every wipe sample [262].

Table S8. Contact angle and surface energy result of wipe samples, data represented the mean of 10 repetitions with three testing liquids: Distilled water (DW), Polyethylene glycol (PEG), and Polyethylene glycerol (Glycerin).

	DW		PEG		Glycerin		Surface energy	Dispersive	Polar	Errors	
Sample name	**CA**	**SD**	**CA**	**SD**	**CA**	**SD**				**RQ**	**sChi**
1.PET	139.1	3.6	27.9	10.0	140.1	8.0	11.58	10.90	0.68	0.00	31.58
2.PET (woven)	93.6	18.7	20.8	4.3	106.7	4.5	25.52	20.65	3.87	0.00	18.35
3.PA (woven)	76.5	6.5	29.7	3.8	101.5	6.3	27.25	12.96	14.29	0.00	18.45
4.CEL/PP	69.3	74.3	47.0	32.0	103.4	9.4	33.08	6.31	29.76	0.39	18.72
5.CEL/PET	0.0	0.0	22.9	7.7	67.0	5.1	96.49	5.78	90.71	0.83	17.40
6.CEL/PET	0.0	0.0	22.7	9.9	118.33	7.1	113.22	0.27	112.95	0.58	37.56
7.Woodpulp/ PET	25.5	54.0	47.0	32.0	105.4	12.0	108.60	0.11	108.49	0.71	27.10
8.Cotton (woven)	0.0	0.0	58.4	7.1	47.7	8.1	123.49	5.53	117.96	0.10	1.17

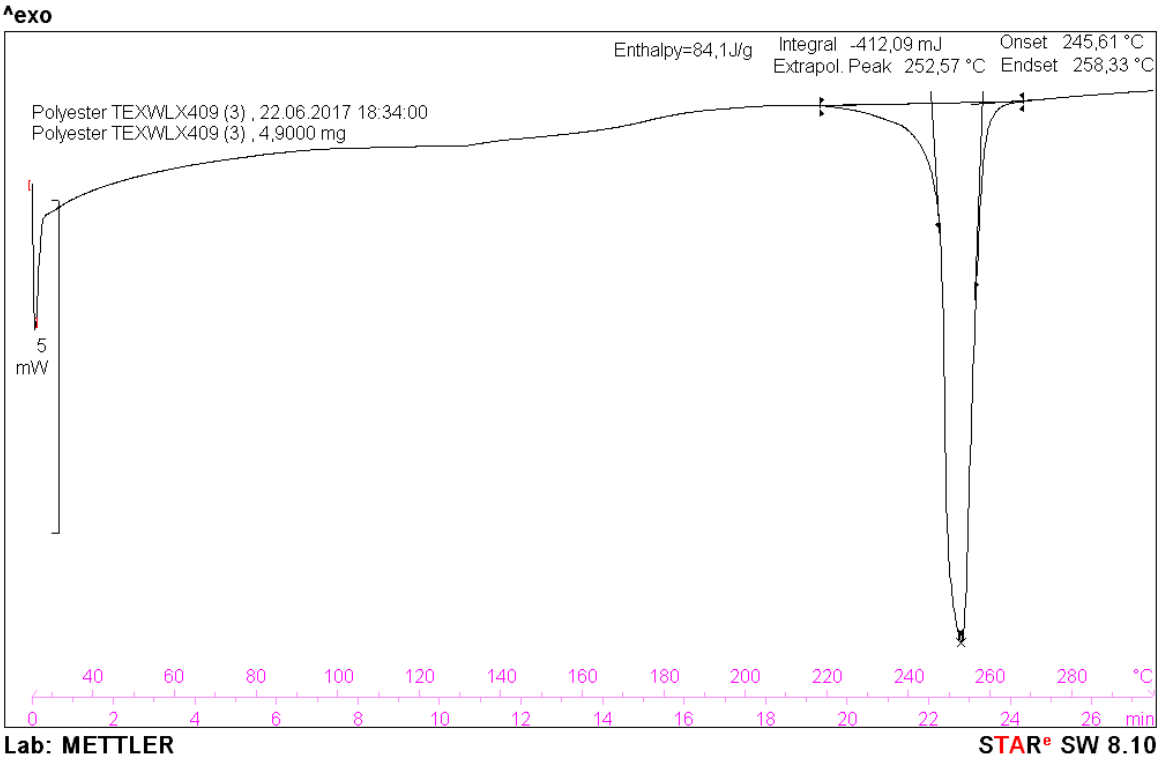


Figure S1. DSC result of wipe sample 1. PET.

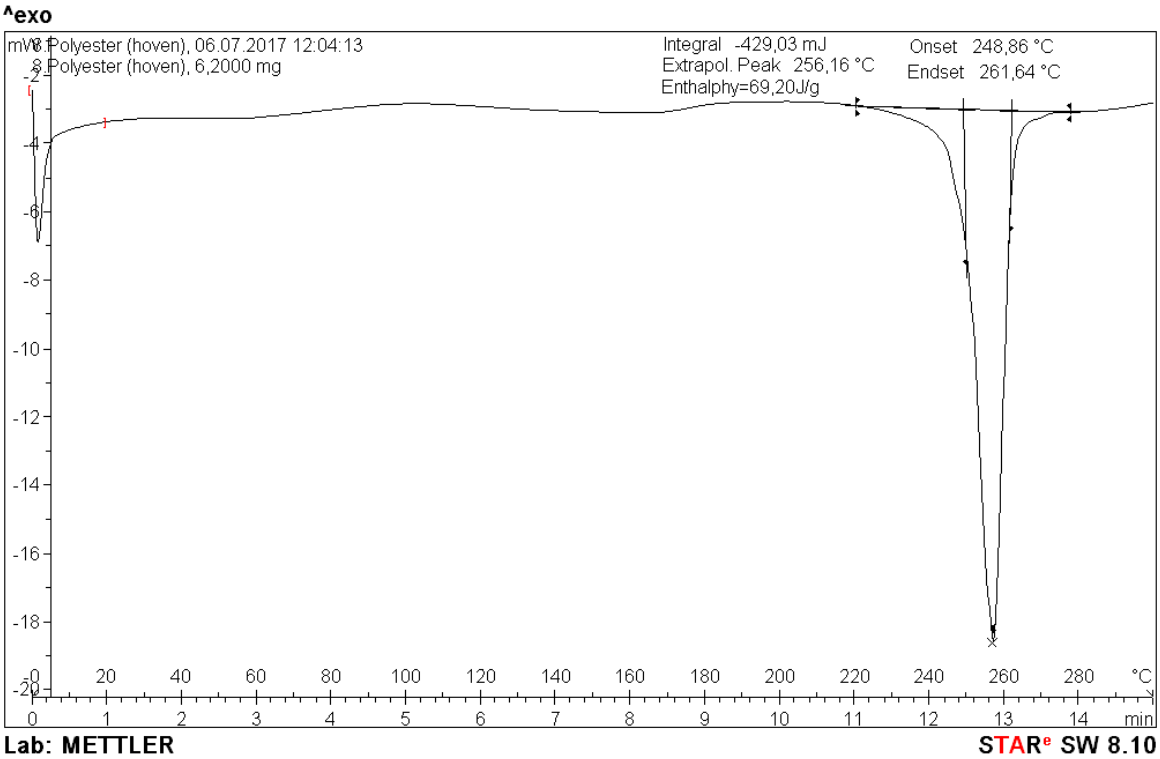


Figure S2. DSC result of wipe sample 2. PET (woven).

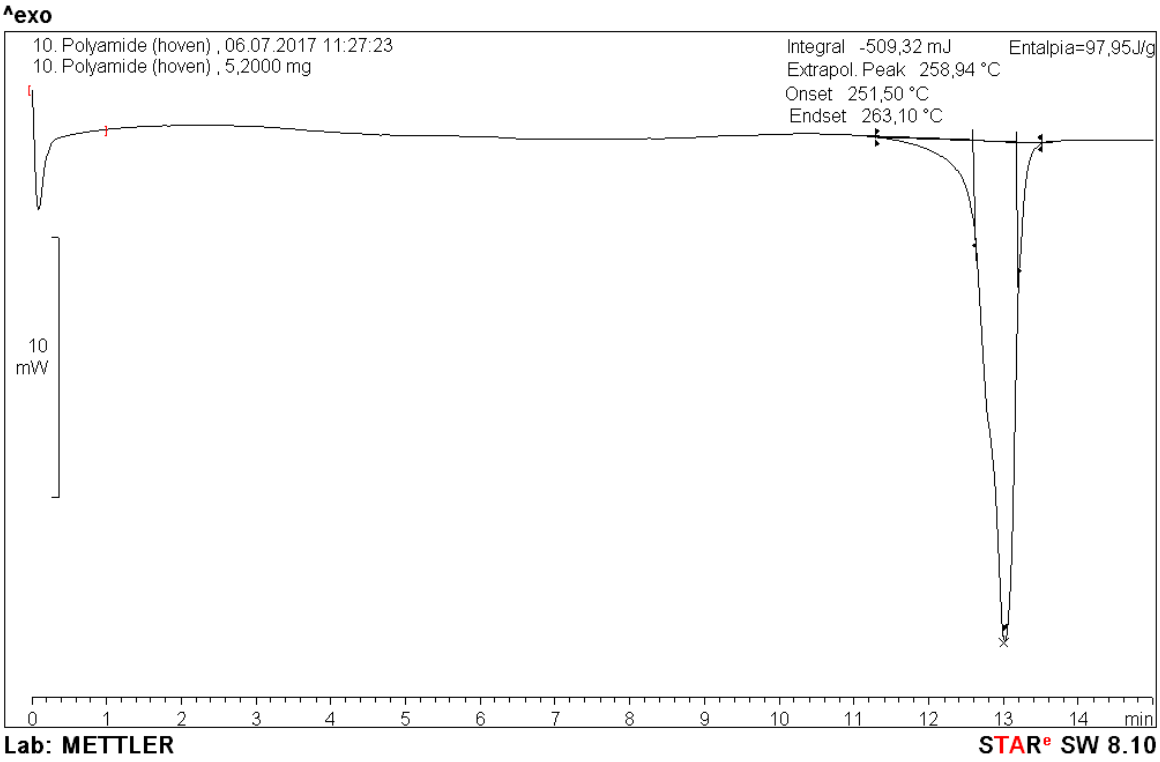


Figure S3. DSC result of wipe sample 3. PA (woven).

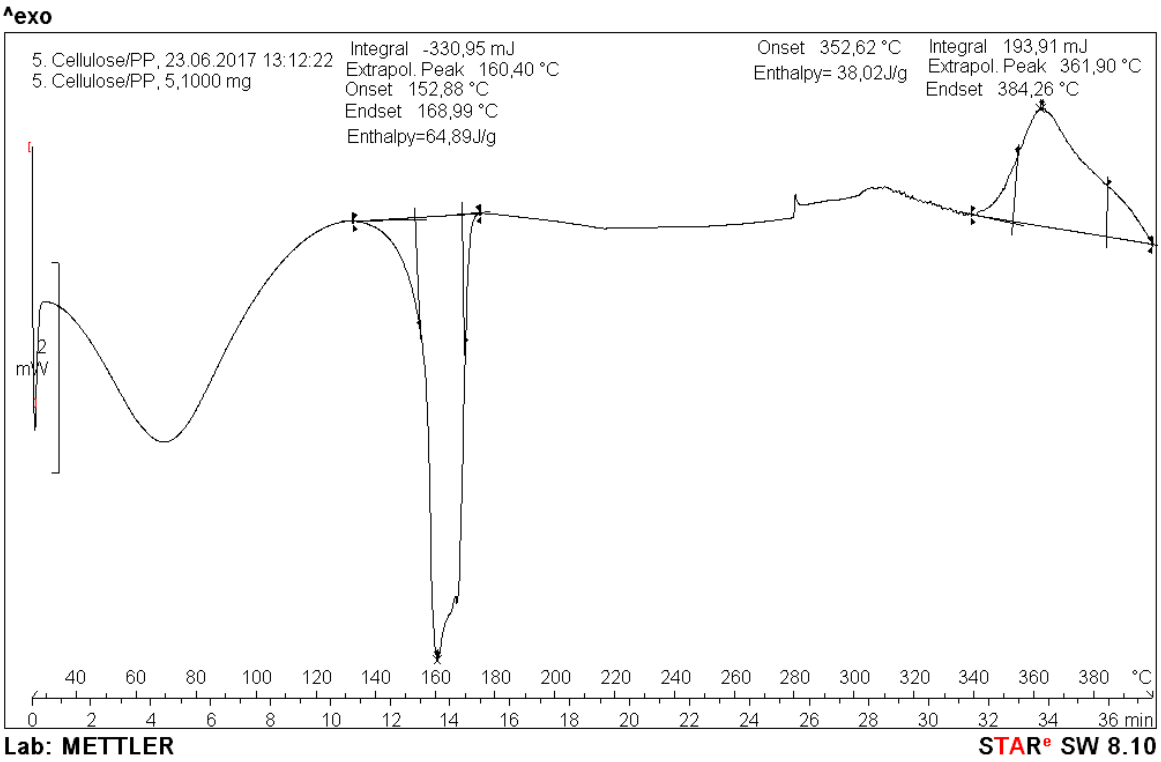


Figure S4. DSC result of wipe sample 4. CEL/PP.

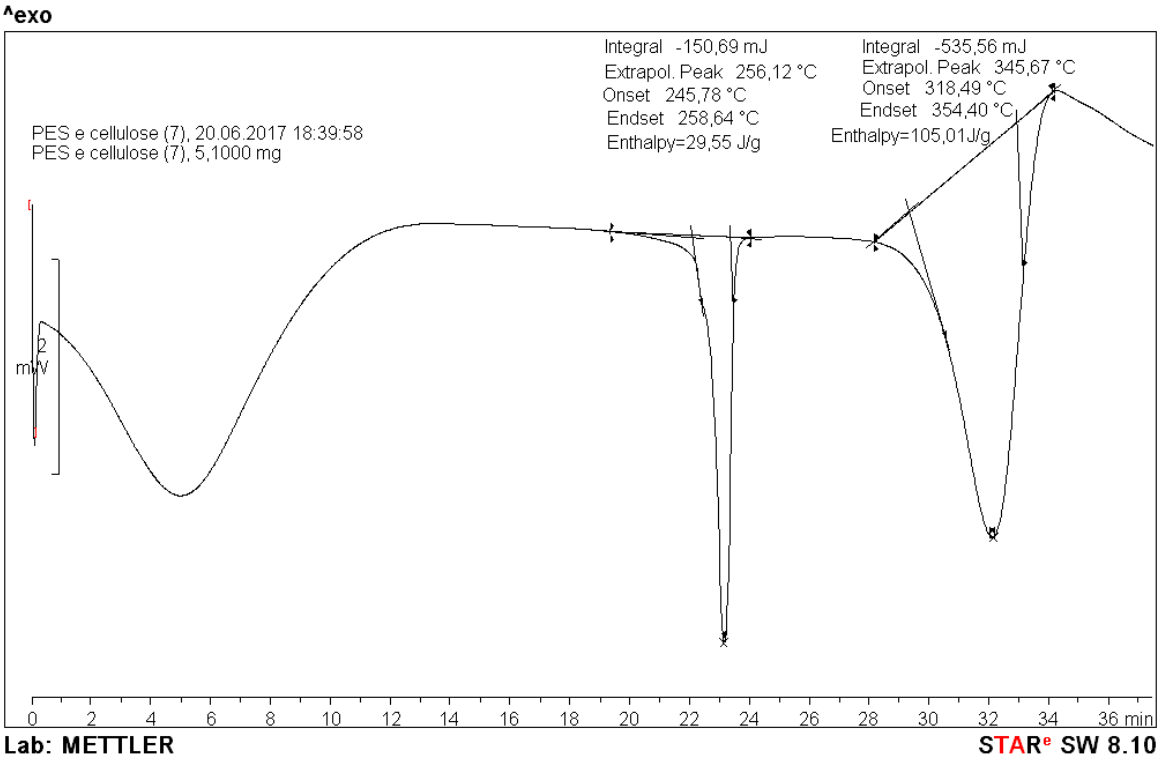


Figure S5. DSC result of wipe sample 5. CEL/PET.

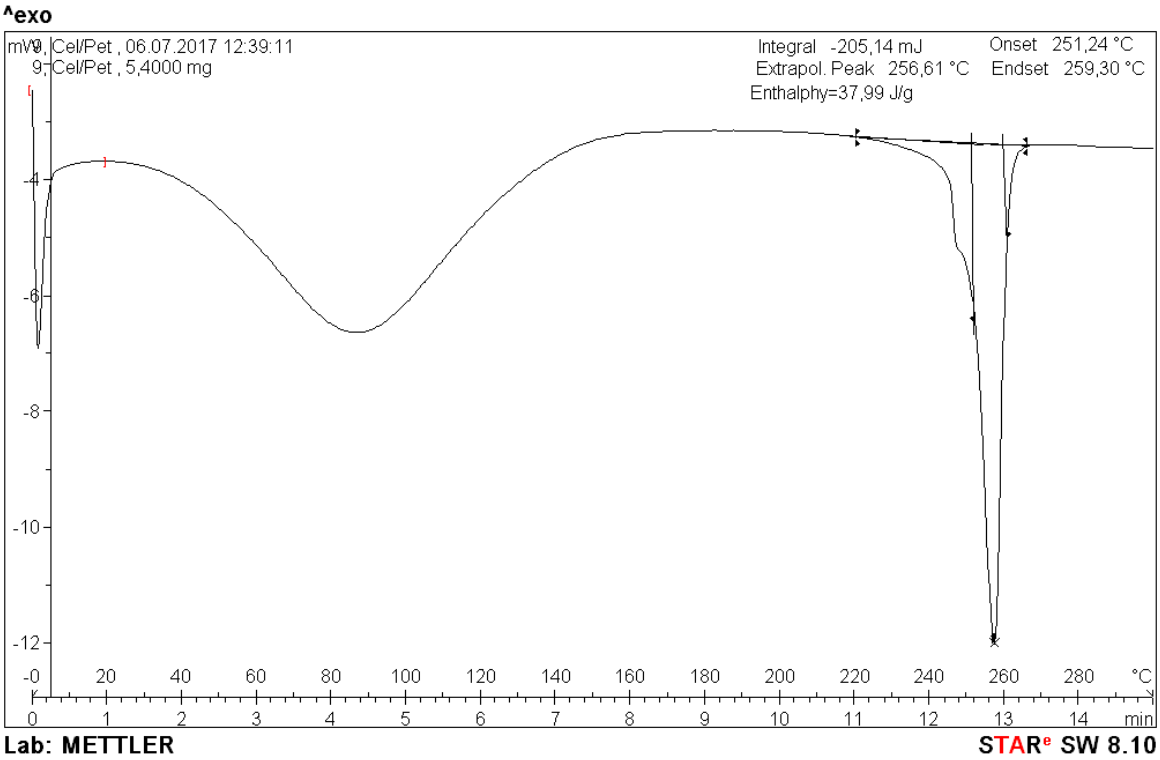


Figure S6. DSC result of wipe sample 6. CEL/PET.

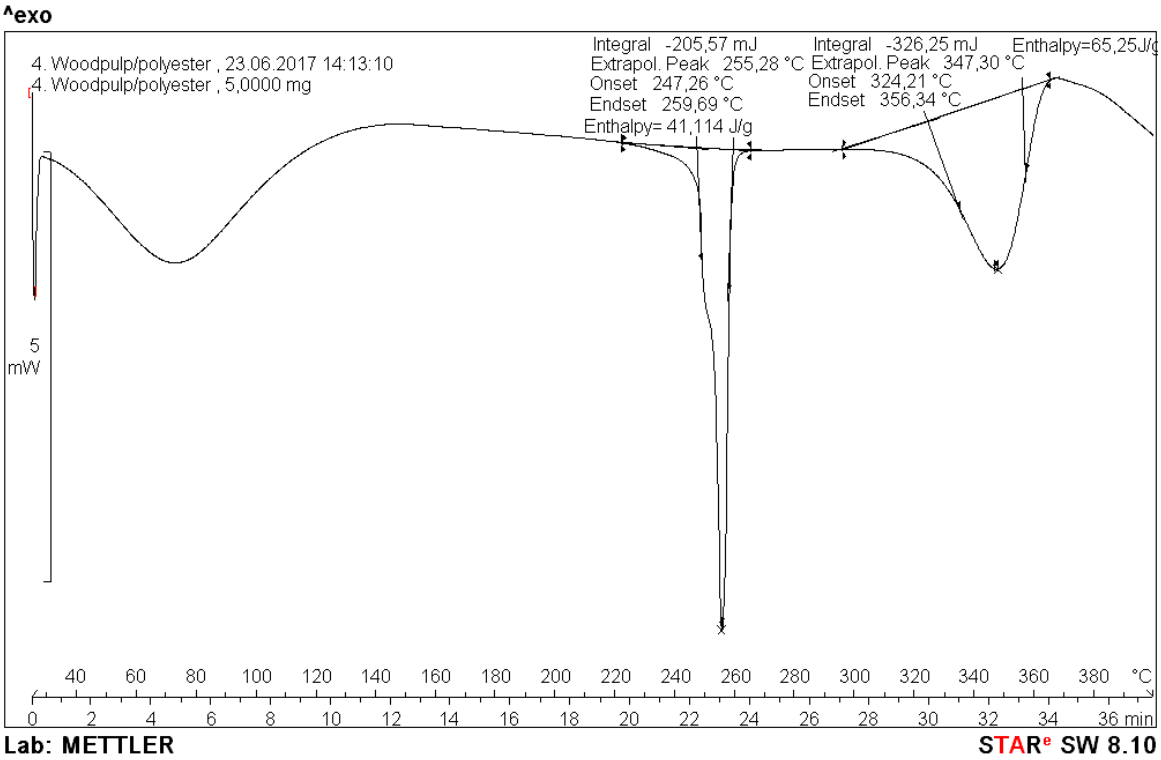


Figure S7. DSC result of wipe sample 7. Woodpulp/PET.

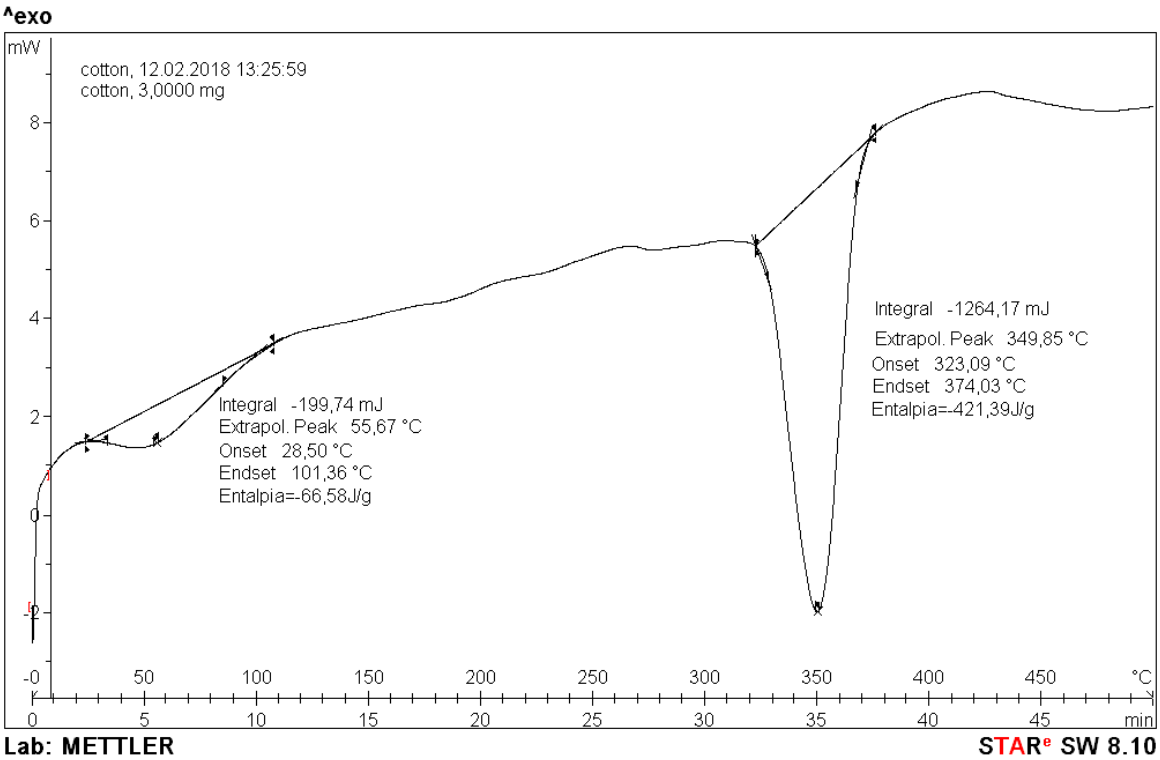


Figure S8. DSC result of wipe sample 8. Cotton (woven).

ANNEX II – Graphical illustration of test procedures

Figure S9. Schematic diagram with a photo of the DBD plasma equipment used for the sample treatment.

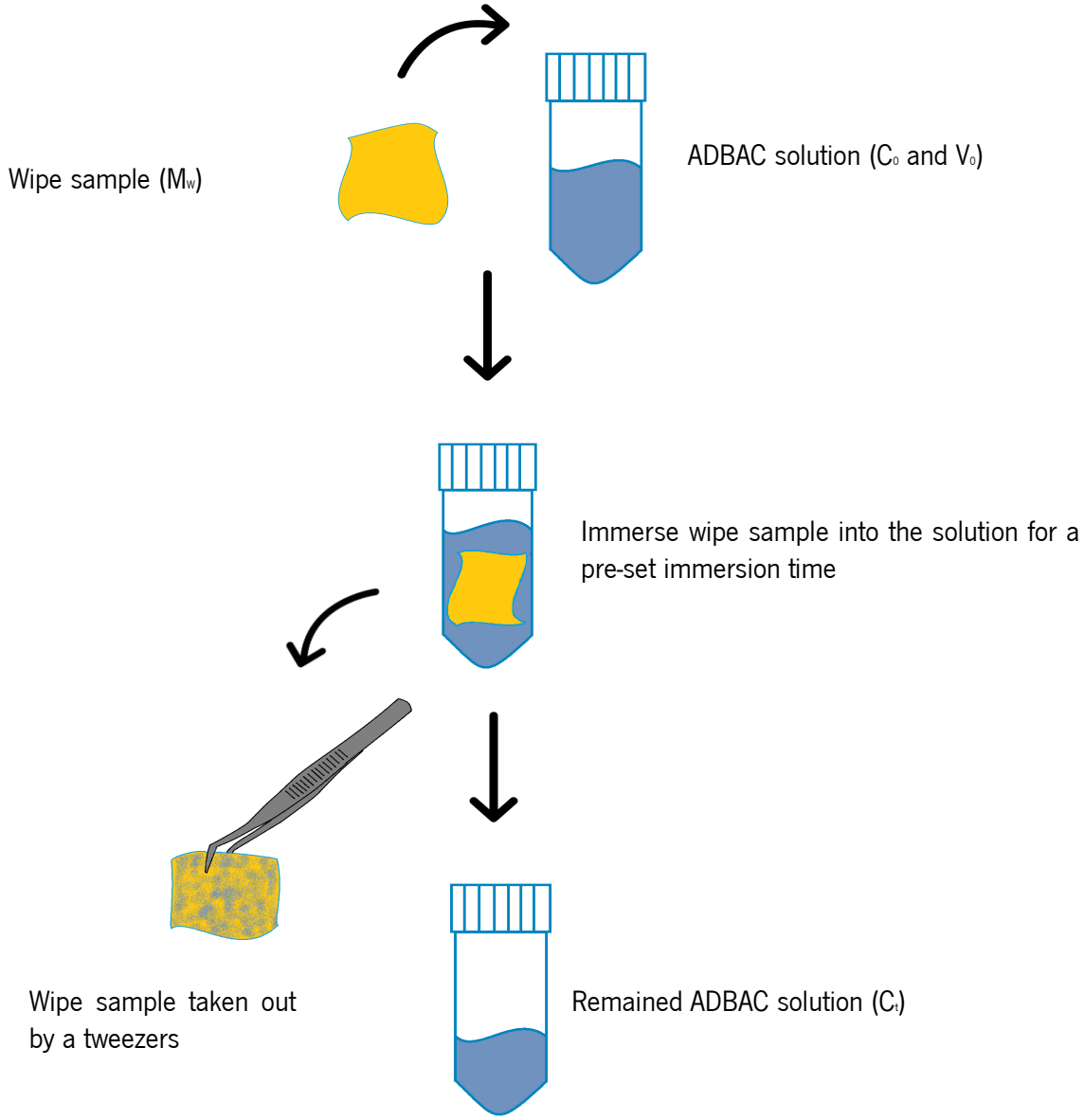
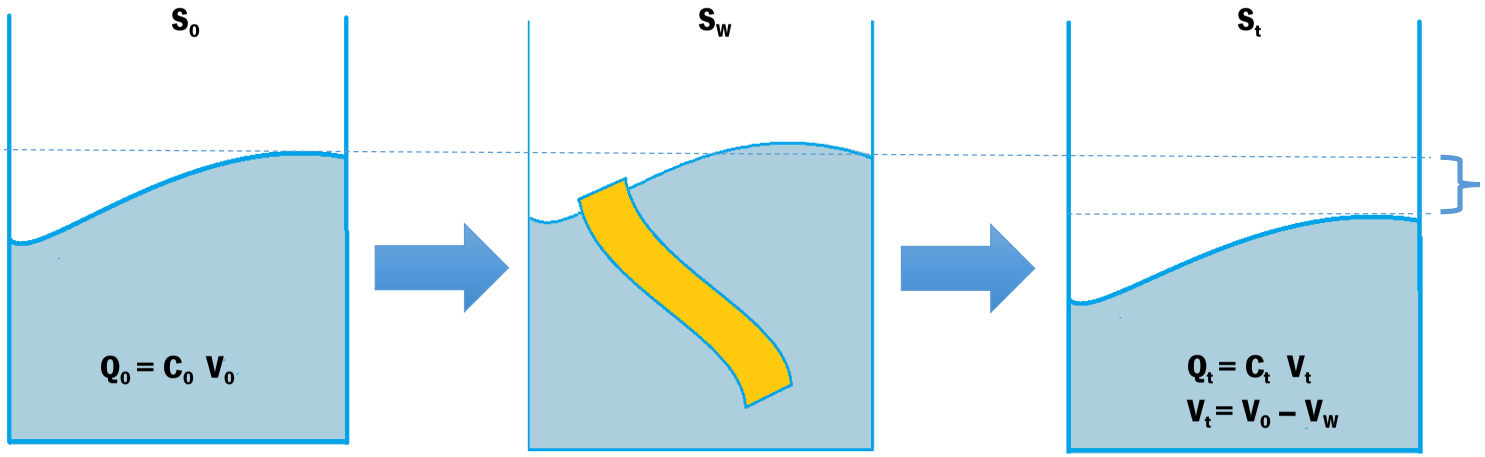


Figure S10. Graphical representation of the immersion process of ADBAC absorption and adsorption test.



Concentration reduction of ADBAC in the bulk solution (C_R)	$C_R = (C_0 - C_t)/ C_0$
Concentration of active ingredients absorbed in the wipe sample (C_Q)	$C_Q = Q_W / V_W$
Weight ratio of active ingredients absorbed in the wipe and wipe mass (R_W)	$R_W = Q_W / M_W$

Note:
C_0 and C_t are calculated from the Abs value measured by UV spectrophotometer based on the Abs vs [C] calibration curve.
Volume and Weight conversion was performed assuming a density of 1 g L^{-1} for the ADBAC solution.

Figure S11. Calculation (absorption and adsorption) illustration.

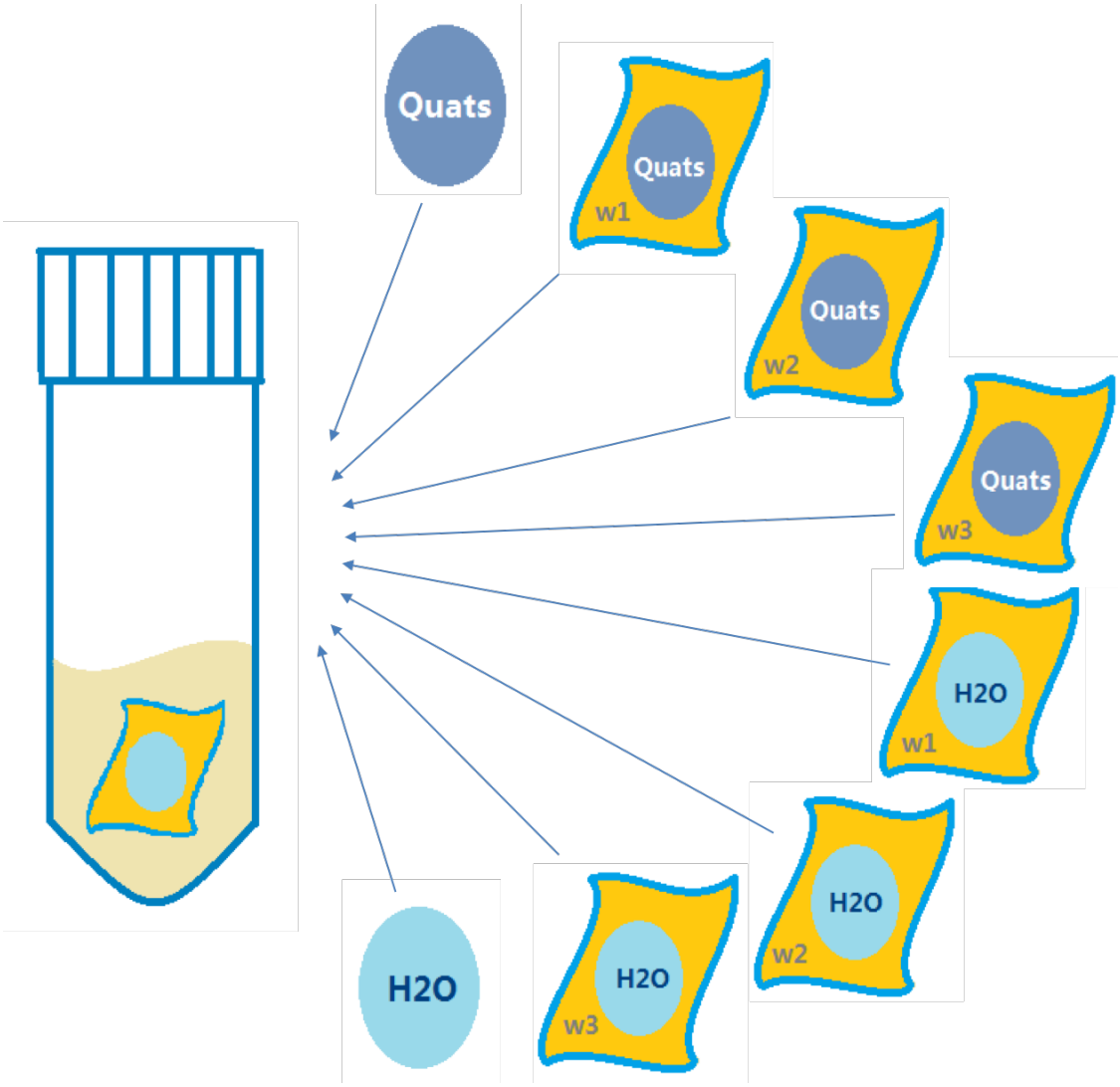


Figure S12. Graphical representation of Shaking Flask Test.

ANNEX III – Supporting Information

Table S9. Concentration Reduction (mean of three repetitions ± SD) of wipe samples in different liquor ratios (LR) as Fabric mass in gram/Solution in mL and immersion time (IT) expressed in minutes.

LR	IT	W1R	W2R	W3R	W1P	W2P	W3P
1:10	**10**	4.25±1.02	19.48±0.78	42.69±4.90	-5.69±2.21	17.15±1.31	25.10±1.61
1:15		4.26±0.39	11.01±1.20	23.48±1.67	5.11±1.19	18.18±1.02	20.74±1.35
1:20		2.96±0.79	12.07±0.78	15.89±0.42	4.30±1.31	18.35±0.53	18.57±0.76
1:25		3.78±0.26	11.98±0.54	18.30±1.88	5.59±0.24	18.73±1.49	16.06±0.55
1:30		1.76±0.49	10.18±1.01	12.90±1.69	1.61±0.11	11.41±0.91	14.36±0.58
1:40		2.61±1.25	7.25±1.25	15.91±0.85	2.30±0.40	9.58±0.31	11.00±0.47
1:60		1.35±0.38	5.76±0.69	11.00±1.42	2.20±0.12	6.75±0.23	8.76±0.16
1:80		1.07±0.43	5.12±0.33	10.46±0.23	0.98±0.12	4.42±0.22	6.42±0.06
1:100		3.27±0.20	5.73±0.30	9.90±1.27	1.97±0.12	5.17±0.04	5.96±1.27
1:120		0.69±0.26	4.70±0.21	6.31±0.37	1.93±0.06	4.67±-0.13	4.51±0.37
1:200		0.56±0.23	3.87±0.16	5.15±0.16	0.40±0.10	2.44±0.41	2.22±1.32
1:10	**30**	4.60±0.18	21.90±2.26	51.49±4.50	-4.68±4.46	15.60±0.79	29.39±1.91
1:15		4.30±0.24	16.03±1.76	27.89±1.76	1.98±3.39	20.63±1.37	26.02±0.52
1:20		3.45±0.05	13.38±0.50	24.16±1.45	2.17±1.29	19.29±0.22	23.17±0.32
1:25		6.36±0.57	13.64±0.44	23.30±2.75	4.23±1.05	19.43±1.27	19.88±0.25
1:30		1.51±0.19	12.95±0.89	18.65±0.54	-0.1±0.32	14.13±1.3	16.53±0.15
1:40		2.22±0.91	7.66±1.33	15.83±0.74	2.98±0.11	9.34±0.16	13.01±0.45
1:60		3.09±0.06	9.29±0.19	15.52±0.99	1.79±0.33	6.09±0.30	10.36±0.48
1:80		2.64±0.22	7.38±0.65	13.82±0.51	1.45±0.21	4.88±0.74	8.44±0.32
1:100		3.08±0.44	6.40±0.51	13.98±1.87	1.53±0.14	5.31±0.08	6.91±1.87
1:120		1.12±0.11	5.21±0.39	7.70±0.51	2.00±0.07	4.47±-0.09	5.73±0.51
1:200		1.08±0.00	4.90±0.33	7.22±0.31	0.43±0.21	3.46±0.37	4.47±0.10
1:10	**60**	9.92±2.20	30.61±1.67	46.55±2.01	-0.43±4.40	21.96±0.31	31.62±1.41
1:15		4.88±0.43	14.29±1.39	36.80±0.08	7.59±2.96	22.28±2.33	24.57±1.54
1:20		4.44±0.37	13.56±1.08	21.82±2.95	2.94±1.01	21.27±0.73	25.78±0.35
1:25		4.02±0.98	12.59±1.23	26.18±1.26	5.49±1.15	17.03±0.28	21.66±0.25

1:30		0.92±0.24	11.46±1.34	18.72±1.27	2.30±0.80	13.42±0.62	18.97±0.36
1:40		8.36±0.19	13.30±2.12	22.44±1.45	1.84±0.35	10.43±0.06	14.42±0.26
1:60		2.05±0.51	7.71±0.44	15.27±0.71	1.39±0.31	7.08±0.87	12.04±0.18
1:80		1.70±0.06	6.45±0.31	12.59±1.75	1.12±0.18	5.18±0.25	9.11±0.31
1:100		5.46±0.33	9.29±0.50	17.39±0.56	1.09±0.15	5.31±0.13	7.35±0.56
1:120		0.95±0.25	5.73±0.26	7.76±0.85	1.15±0.09	4.33±-0.05	6.27±0.85
1:200		1.31±0.20	5.28±0.48	7.70±0.42	0.43±0.38	3.12±0.21	4.33±0.15
1:10	**90**	3.62±0.65	22.31±0.91	59.27±2.27	1.15±3.67	21.99±1.24	33.50±1.17
1:15		7.26±0.06	16.23±3.13	37.44±1.22	6.59±1.35	22.18±4.62	30.03±1.35
1:20		1.70±0.83	14.02±1.72	23.60±0.12	2.83±1.41	21.21±1.45	26.89±0.61
1:25		5.06±0.74	14.43±0.33	28.87±0.48	2.87±0.11	17.41±0.78	22.56±0.30
1:30		3.88±1.00	12.92±2.29	21.92±0.58	0.43±0.11	13.32±0.78	19.07±0.30
1:40		3.82±0.98	10.01±0.95	20.93±2.36	2.66±0.26	11.31±0.62	14.95±0.41
1:60		0.62±0.45	6.84±0.60	15.45±1.26	2.33±0.46	7.67±0.26	12.49±0.22
1:80		2.64±0.06	7.28±0.54	13.75±0.91	0.57±0.38	5.96±0.44	9.56±0.22
1:100		2.77±1.02	5.32±1.10	13.71±1.76	1.76±0.17	5.81±0.17	8.19±1.76
1:120		1.02±0.20	5.14±0.62	8.88±0.51	1.09±0.11	4.77±0.00	6.70±0.51
1:200		1.67±0.10±	5.01±0.16	8.20±0.06	0.70±0.10	3.25±0.10	4.20±0.25

Table S10. Two-way ANOVA analysis results of W1pet, W2CEL/PET, W3cotton (R), and their plasma-treated samples (P) with Immersion time (IT) and Liquor ratio (LR) as the factors at significant level 0.05.

Source of Variation		SS	df	MS	F	P-value	F crit
ANOVA result of W1R pet	**IT**	14.06946	3	4.689821	2.178932	0.11116	2.922277
	LR	110.4017	10	11.04017	5.12936	0.000229	2.16458
	Error	64.57046	30	2.152349			
	Total	189.0416	43				
ANOVA result of W2R CEL/PET	**IT**	54.2839	3	18.09463	6.019073	0.002455	2.922277
	LR	1201.513	10	120.1513	39.96763	1.35E-14	2.16458
	Error	90.18647	30	3.006216			
	Total	1345.984	43				
ANOVA result of W3R cotton	**IT**	309.7265	3	103.2422	15.91979	2.23E-06	2.922277
	LR	5913.166	10	591.3166	91.18016	1.21E-19	2.16458
	Error	194.5544	30	6.485145			
	Total	6417.447	43				
ANOVA result of W1P pet	**IT**	6.443566	3	2.147855	1.113969	0.358911	2.922277
	LR	170.3022	10	17.03022	8.832594	1.51E-06	2.16458
	Error	57.84332	30	1.928111			
	Total	234.5891	43				
ANOVA result of W2P CEL/PET	**IT**	18.38066	3	6.126888	4.063636	0.015501	2.922277
	LR	1941.606	10	194.1606	128.7763	8.13E-22	2.16458
	Error	45.23206	30	1.507735			
	Total	2005.219	43				
ANOVA result of W3P cotton	**IT**	148.9918	3	49.66394	31.08172	2.46E-09	2.922277
	LR	3074.98	10	307.498	192.4448	2.26E-24	2.16458
	Error	47.93551	30	1.59785			
	Total	3271.907	43				

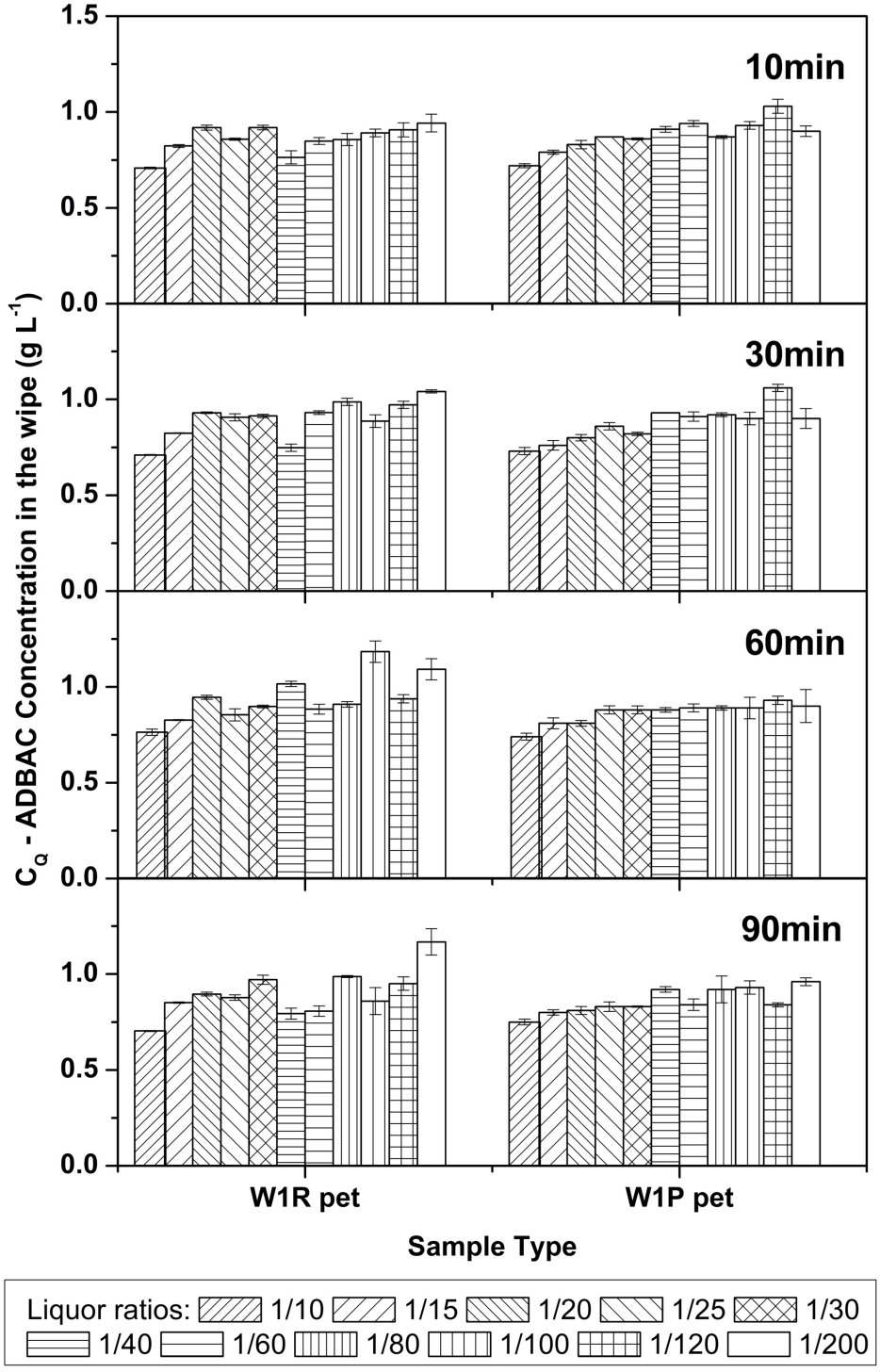


Figure S13. Concentration of ADBAC absorbed in the wipe (C_Q) ± SD of untreated (R) and plasma-treated (P) W1 samples changing with immersion time and liquor ratio.

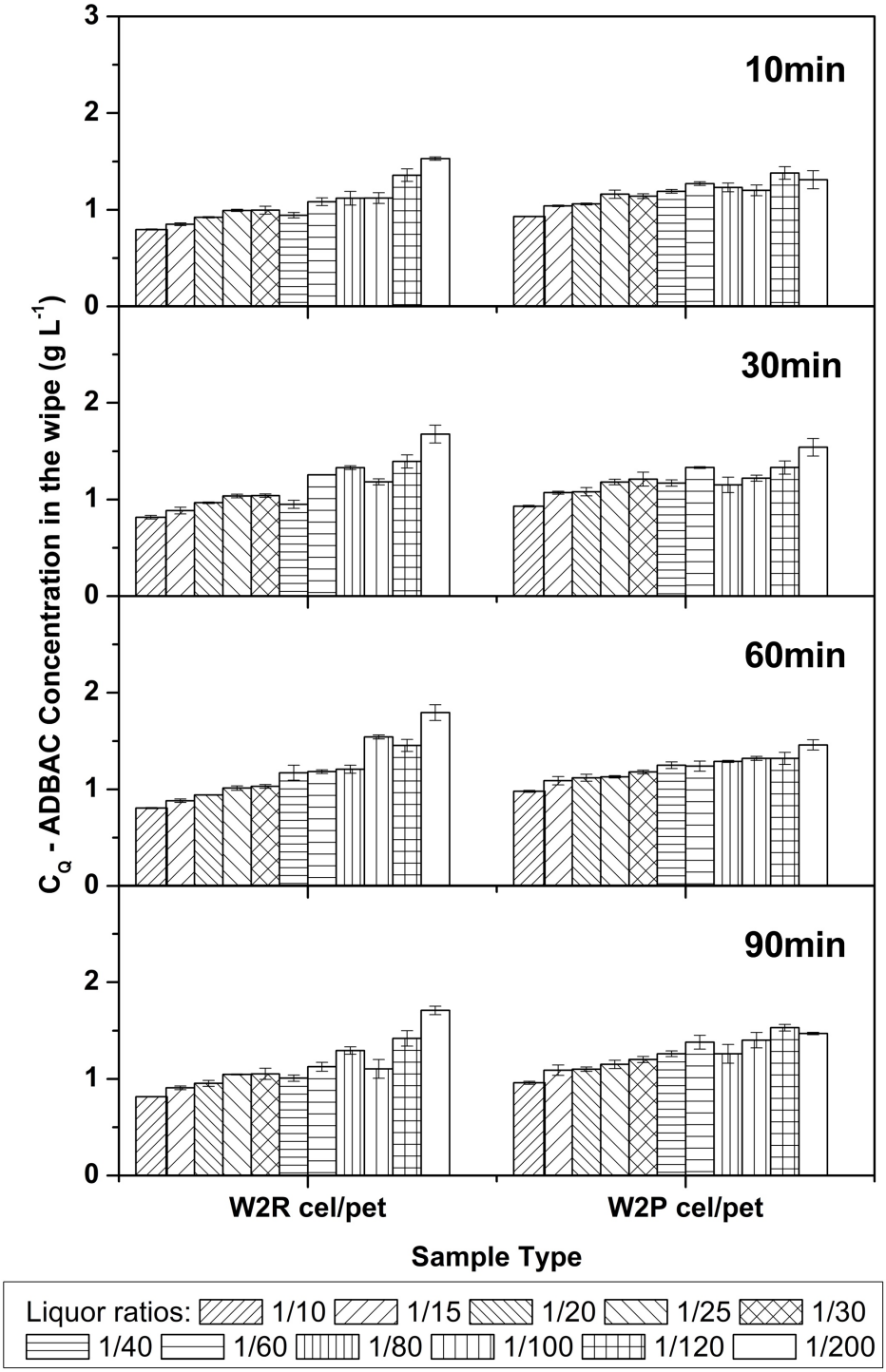


Figure S14. Concentration of ADBAC absorbed in the wipe (C_0) ± SD of untreated (R) and plasma-treated (P) W2 samples changing with immersion time and liquor ratio.

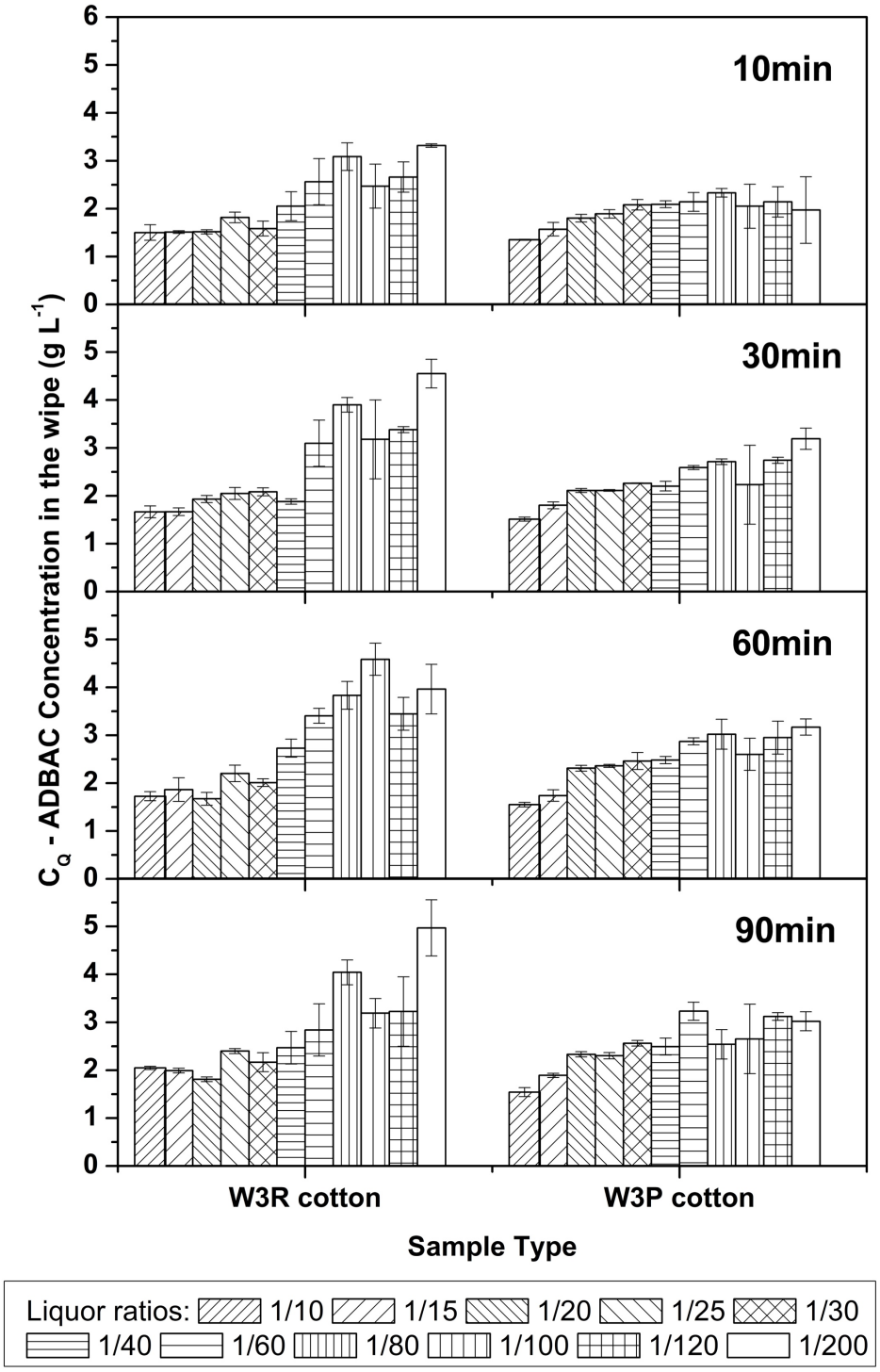


Figure S15. Concentration of ADBAC absorbed in the wipe (C_Q) ± SD of untreated (R) and plasma-treated (P) W3 samples changing with immersion time and liquor ratio.

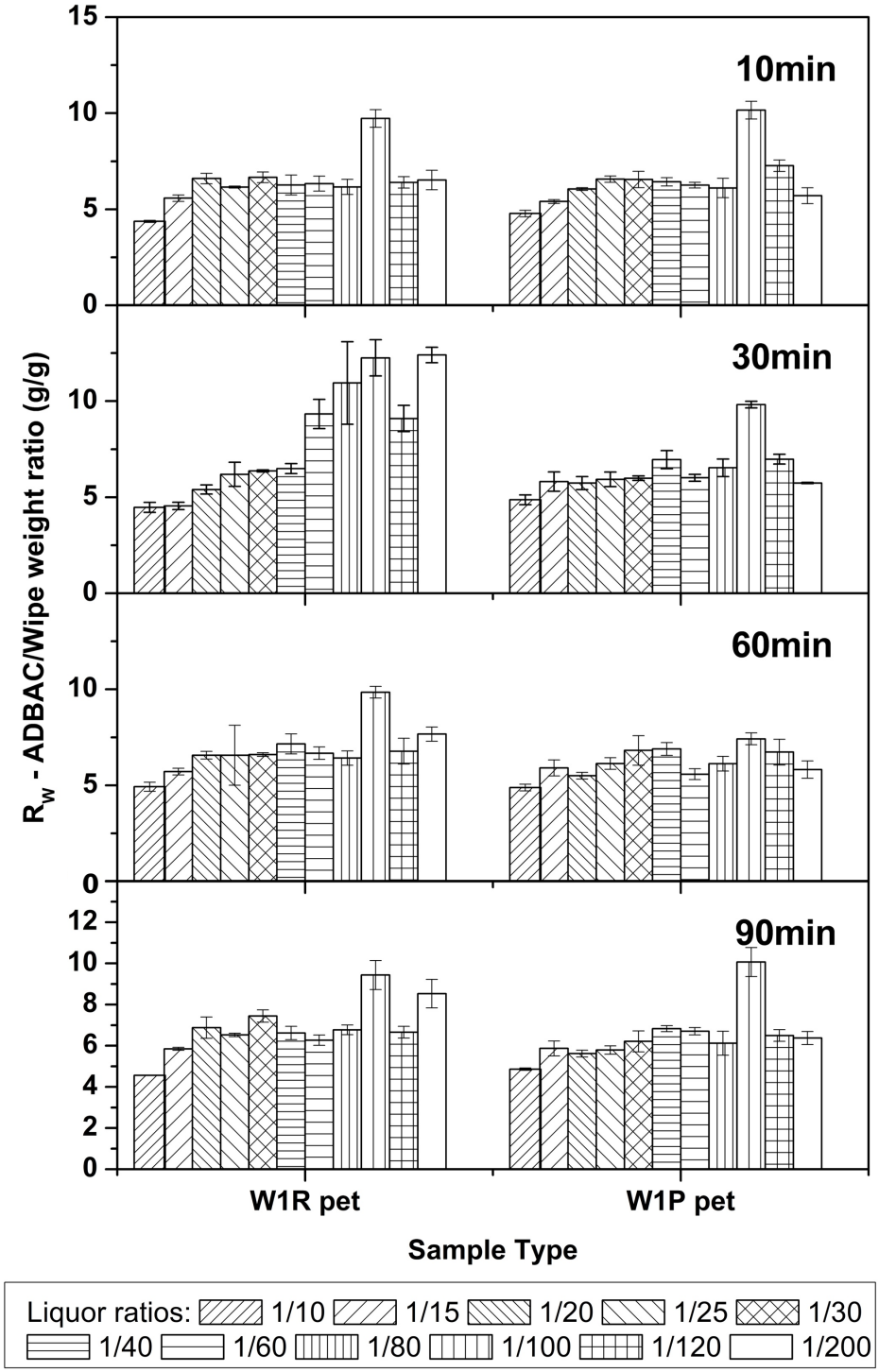


Figure S16. Weight ratio between the amount of ADBAC on the wipe and the wipe mass (R_w) ± SD of untreated (R) and plasma-treated (P) W1 samples changing with immersion time and liquor ratio.

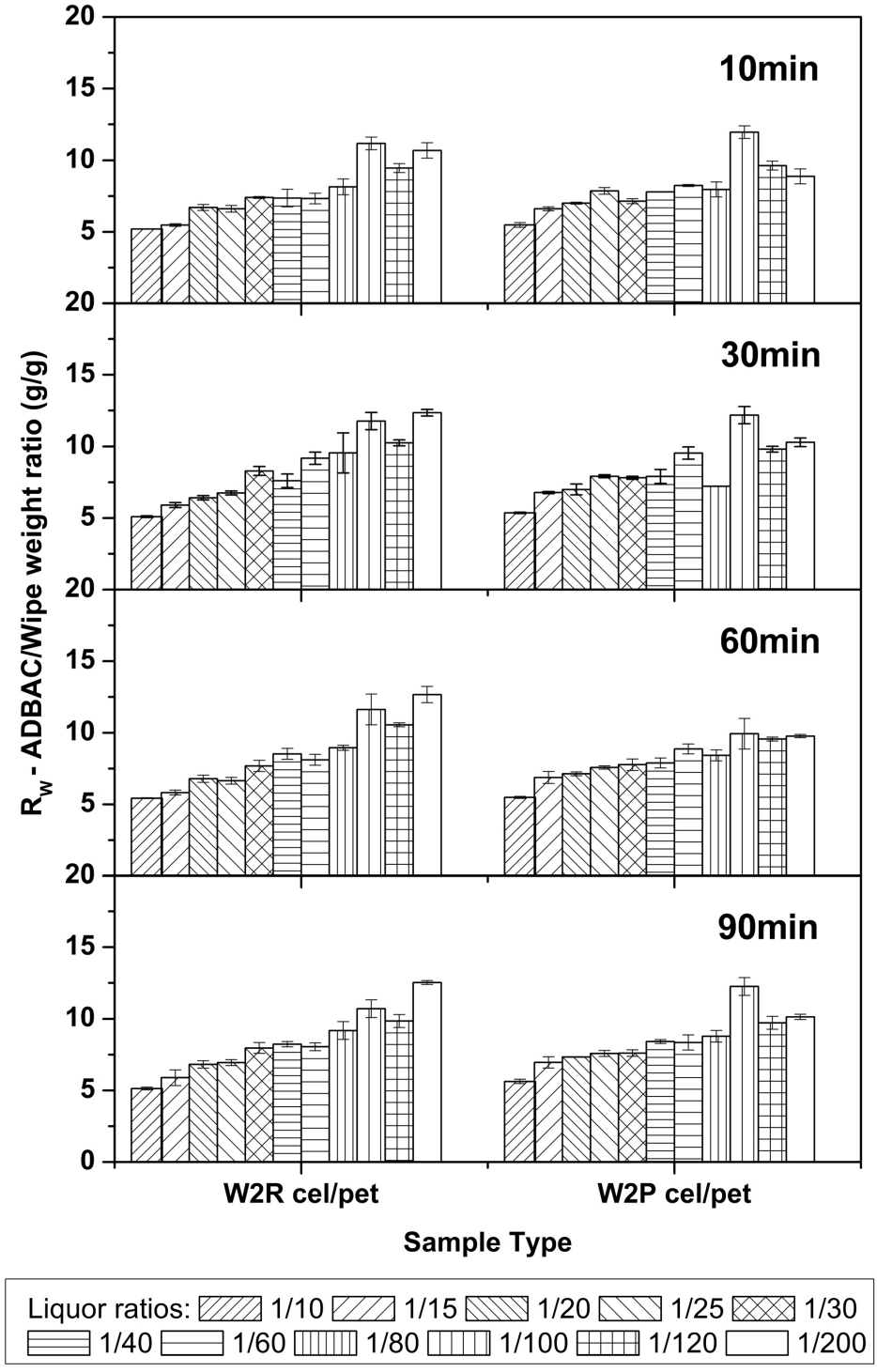


Figure S17. Weight ratio between the amount of ADBAC on the wipe and the wipe mass (R_w) ± SD of untreated (R) and plasma-treated (P) W2 samples changing with immersion time and liquor ratio.

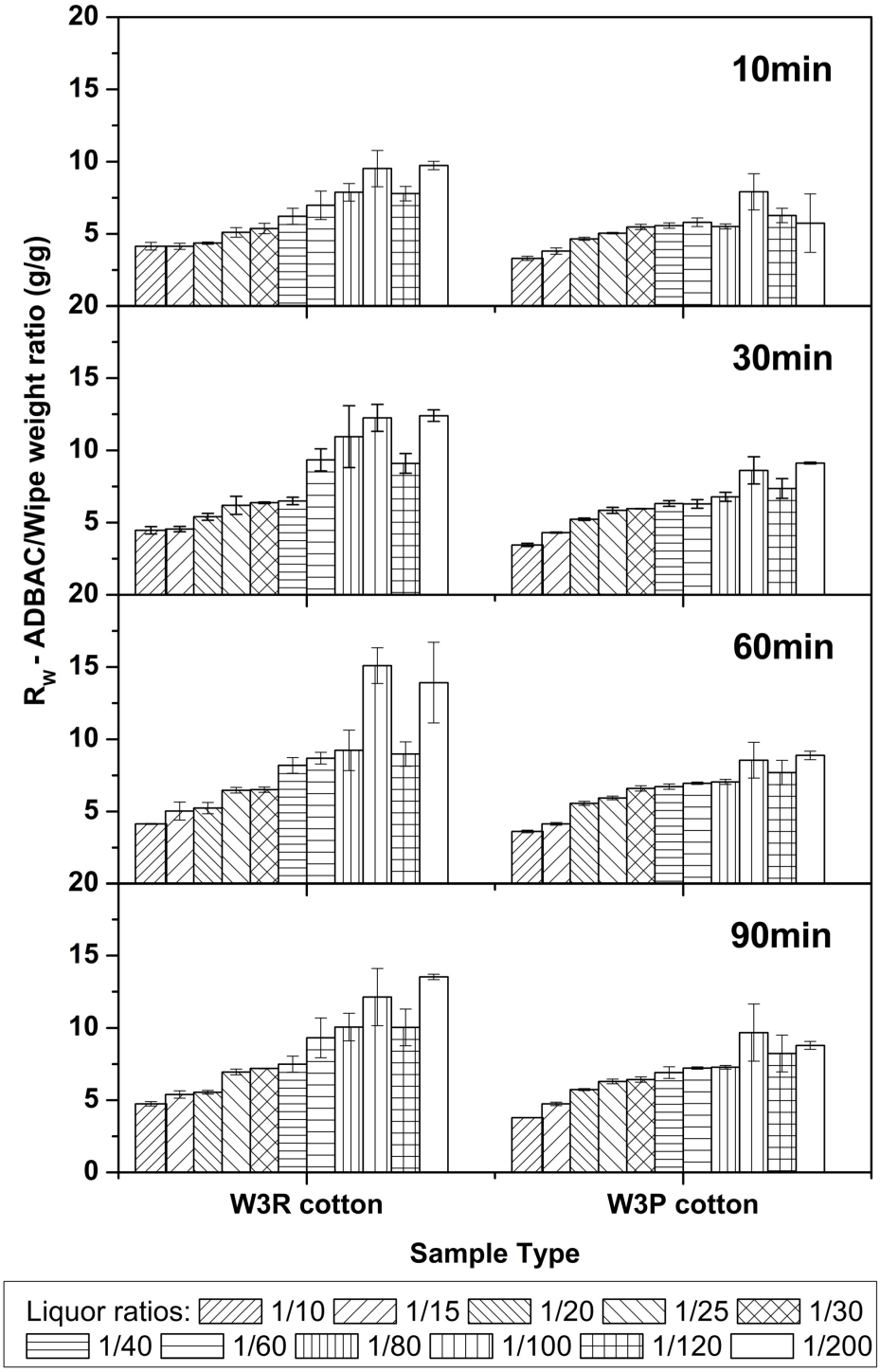
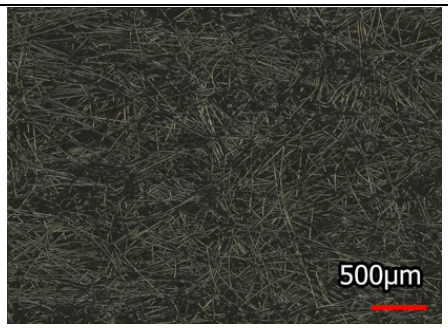
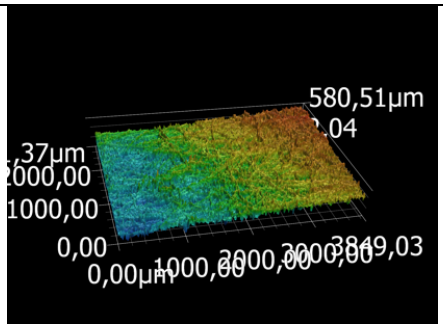
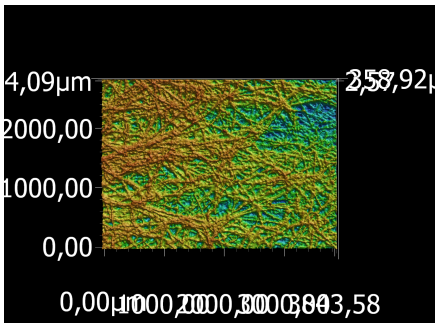
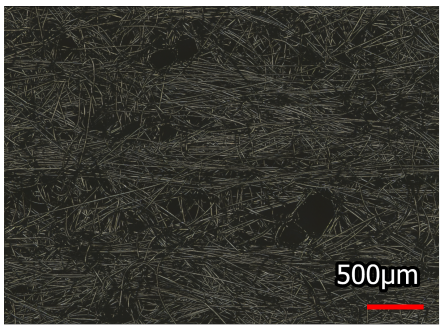
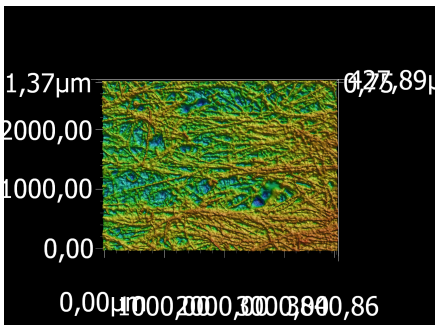
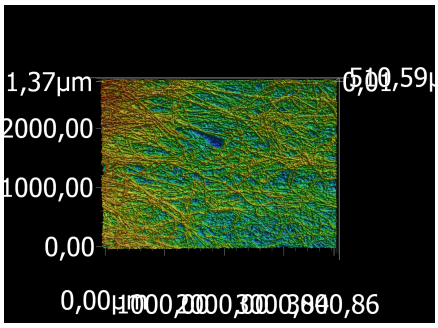
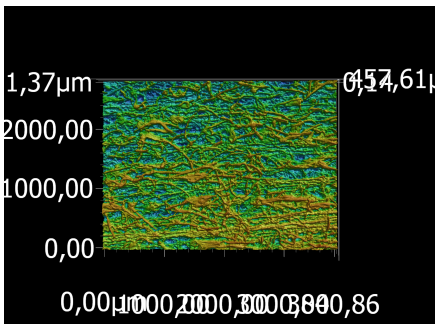


Figure S18. Weight ratio between the amount of ADBAC on the wipe and the wipe mass (R_w) ± SD of untreated (R) and plasma-treated (P) W3 samples changing with immersion time and liquor ratio.

Sample	Laser Optical	3D-Image
W1R front		
W1R back		
W1P front		
W1P back		
W2R front		

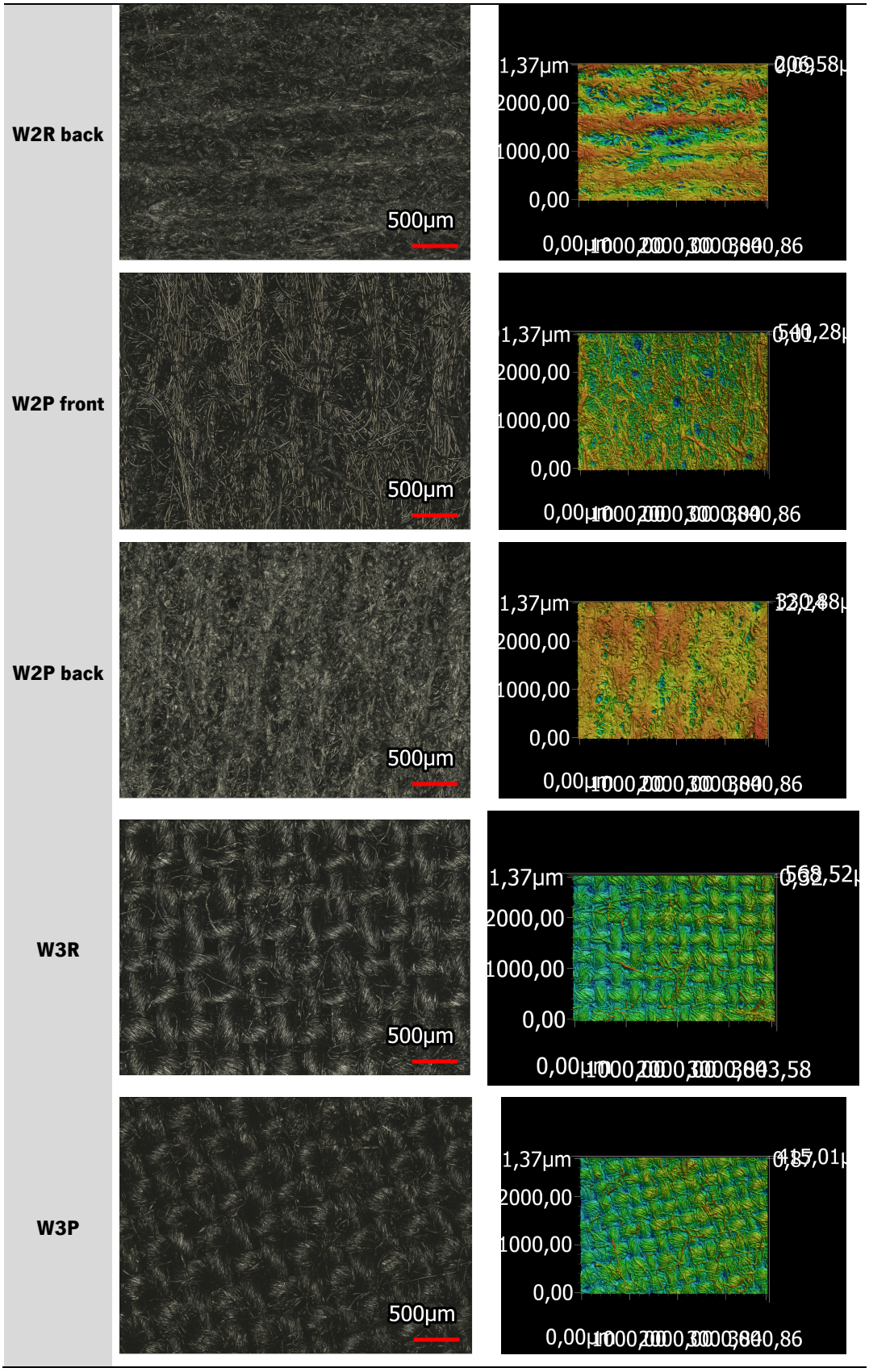


Figure S19. Laser Scanning Microscopic Optical and 3D Images of the untreated (R) and plasma-treated (P) wipes in both sides (only for nonwovens).

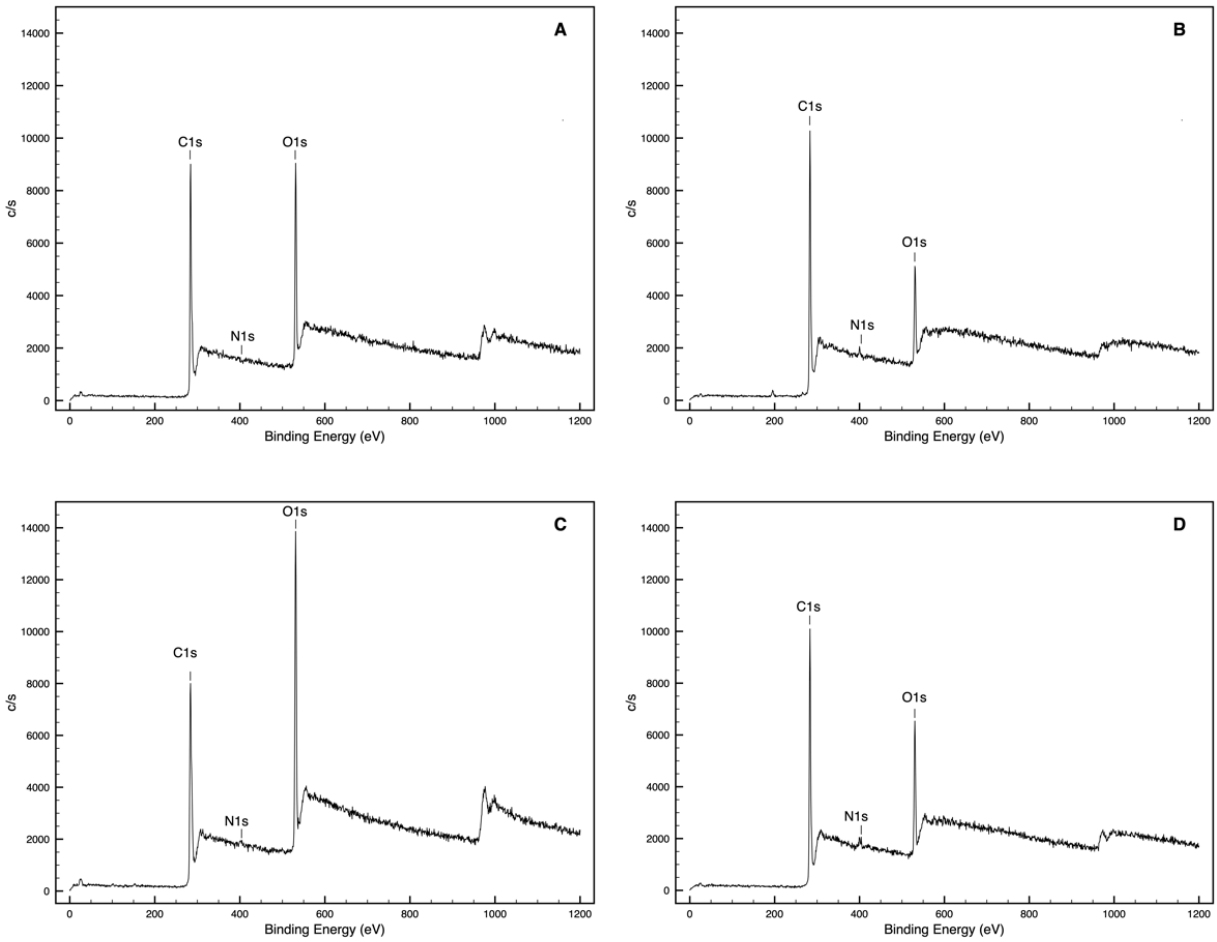


Figure S20. XPS survey scan of polyester nonwoven wipe (W1), A) W1R, B) W1RQ, C) W1P, D) W1PQ.

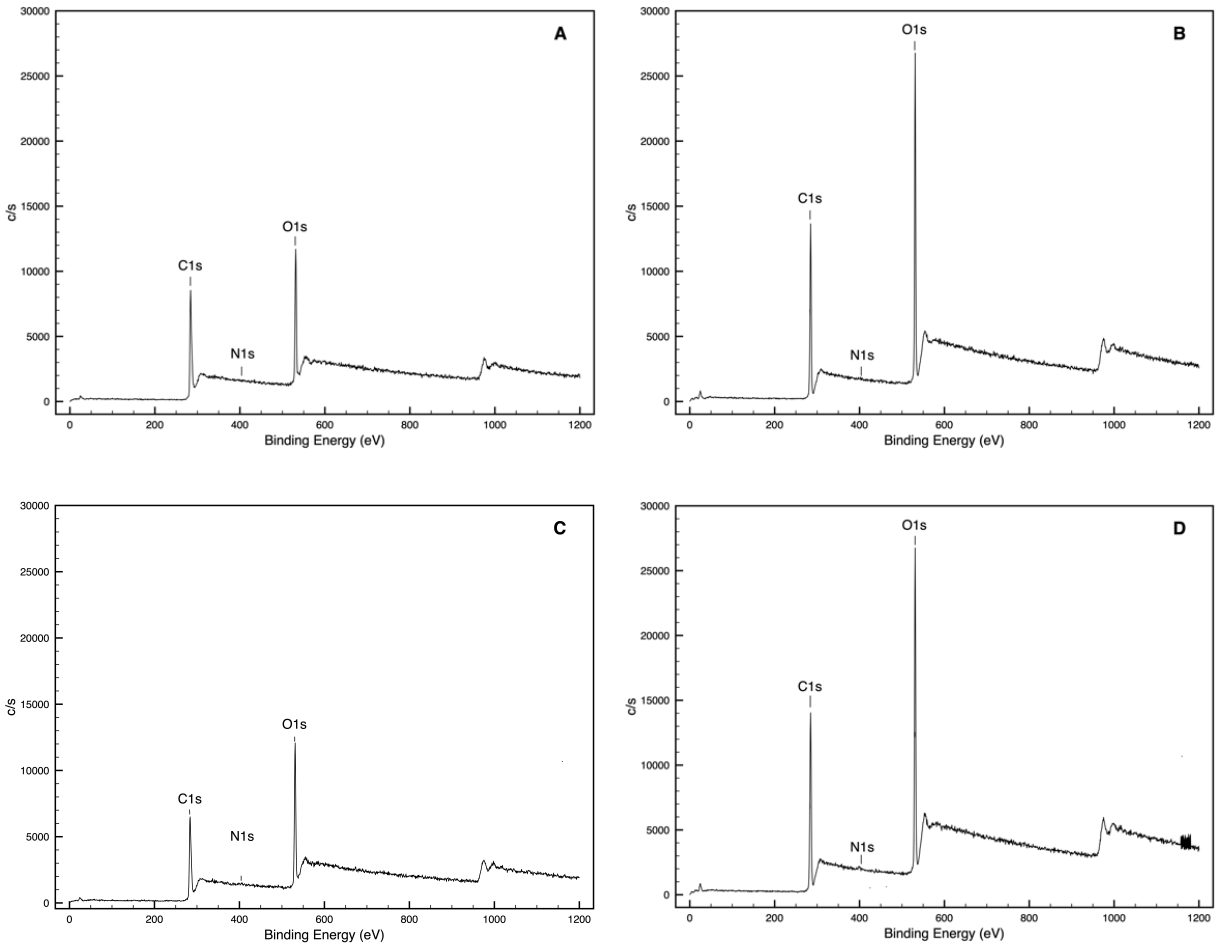


Figure S21. XPS survey scan of polyester/cellulose nonwoven wipe (W2), A) W2R, B) W2RQ, C) W2P, D) W2PQ.

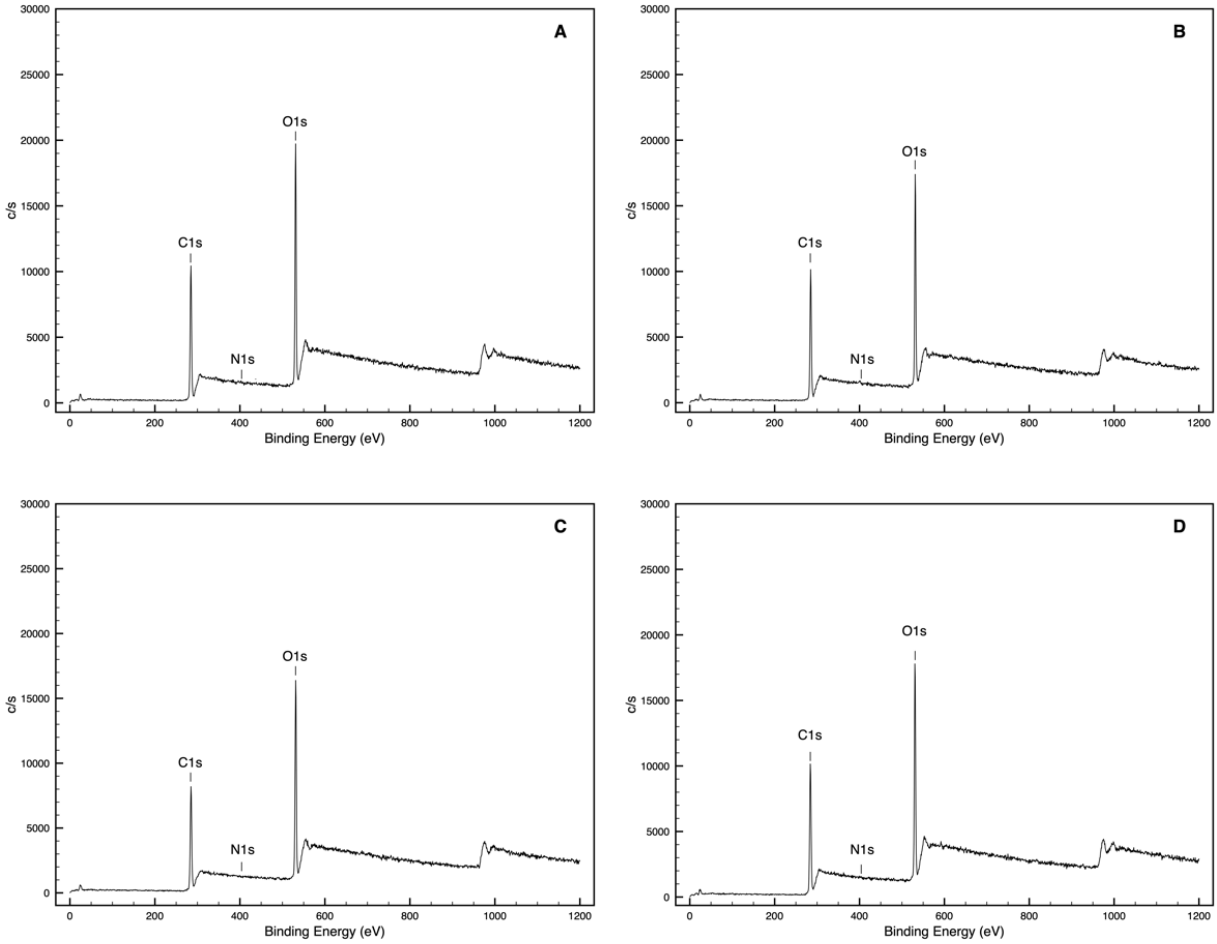


Figure S22. XPS survey scan of woven cotton wipe (W3), A) W3R, B) W3RQ, C) W3P, D) W3PQ.

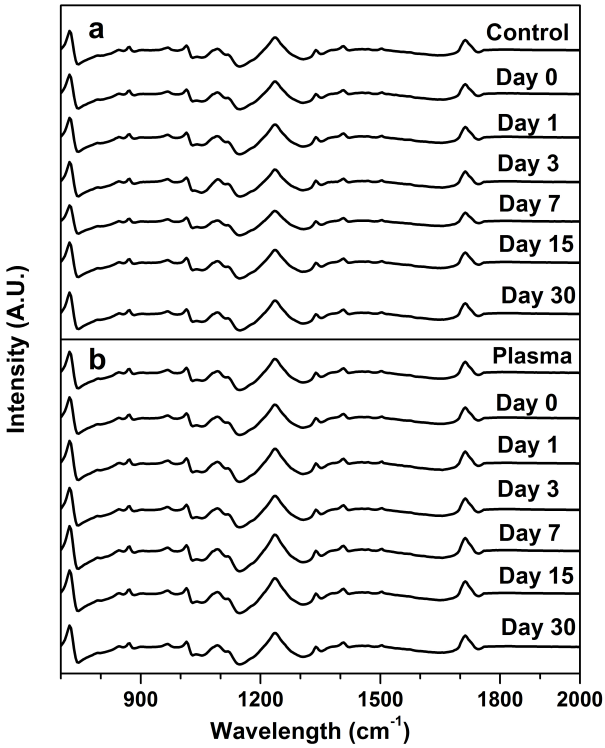


Figure S23. ATR-FTIR spectra of control (a) and plasma-treated (b) W1 (polyester) immersed in ADBAC in the range between 700 and 2000 cm^{-1}.

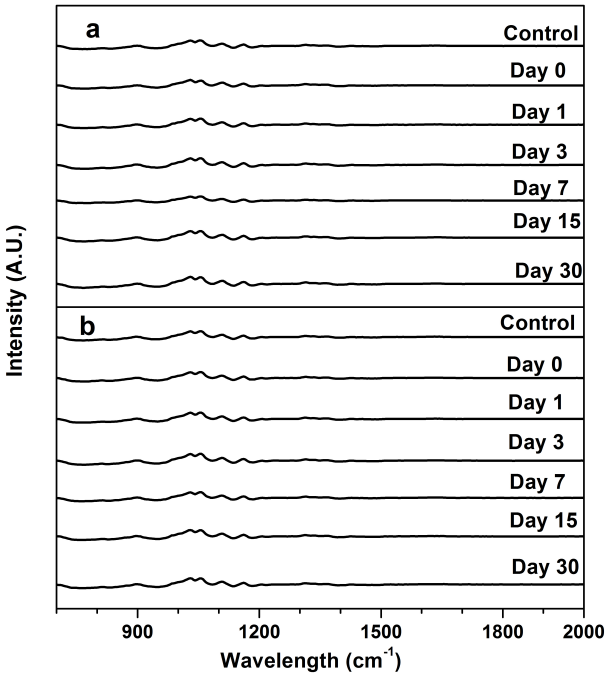


Figure S24. ATR-FTIR spectra of control (a) and plasma-treated (b) W2 (polyester/cotton) immersed in ADBAC in the range between 700 and 2000 cm^{-1}.

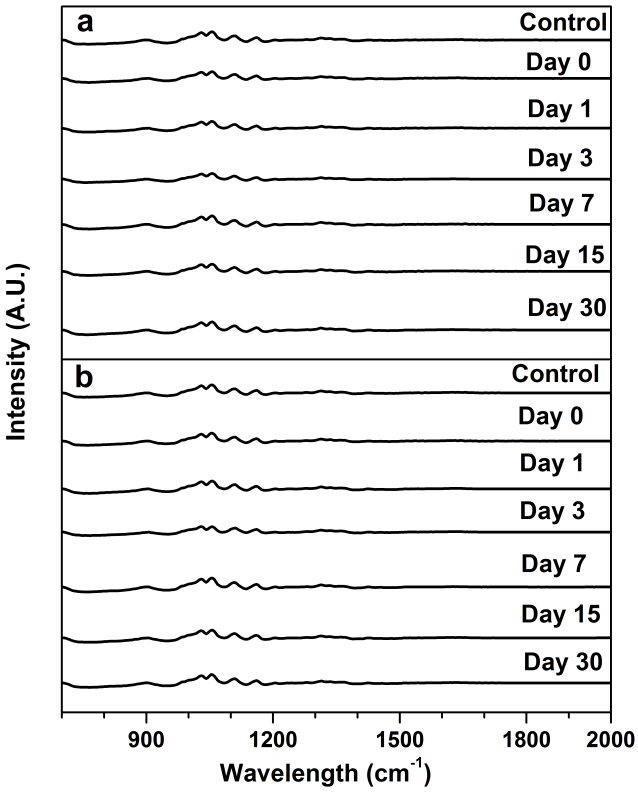


Figure S25. ATR-FTIR spectra of control (a) and plasma-treated (b) W3 (cotton) immersed in ADBAC in the range between 700 and 2000 cm^{-1}.

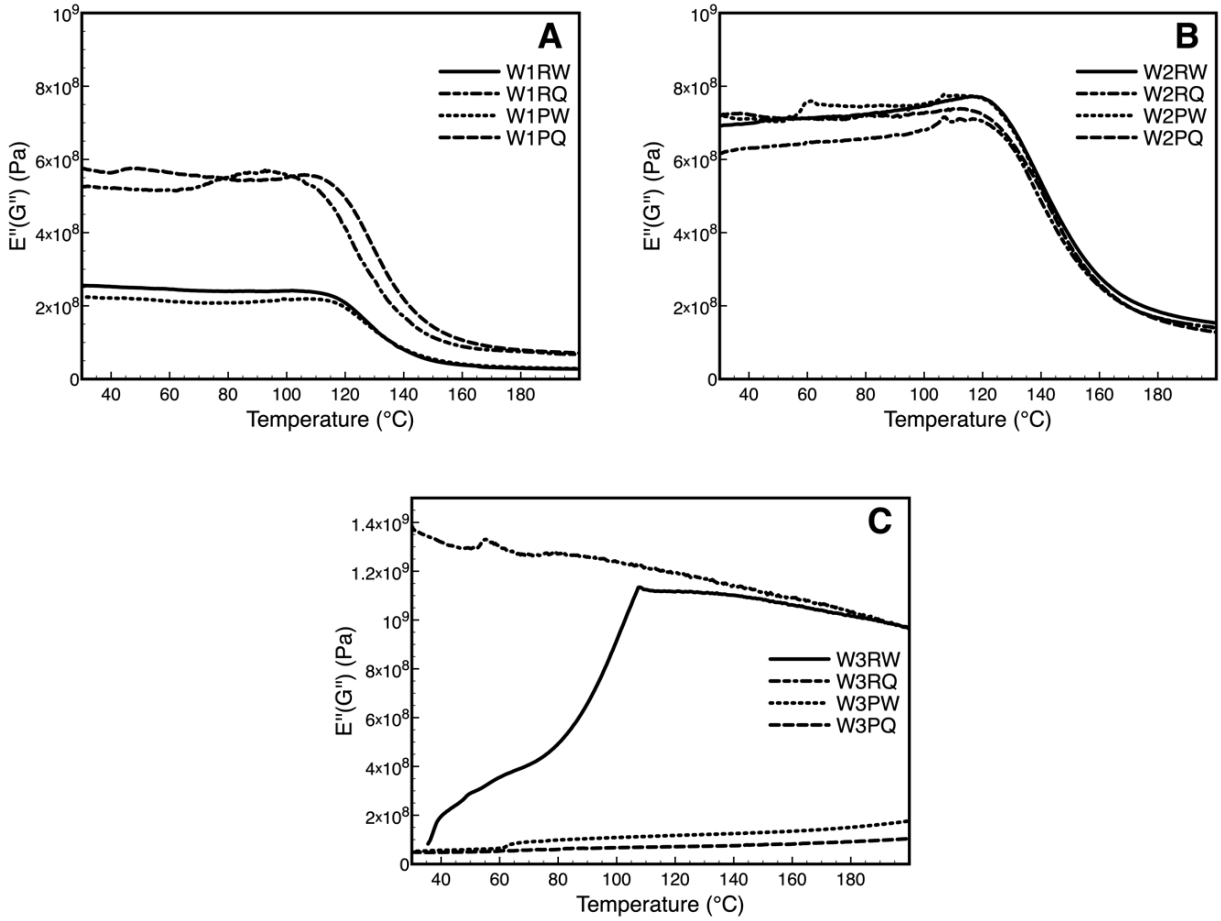


Figure S26. Temperature dependence at 4 Hz of loss (E'') modulus of W1 (A), W2 (B), W3 (C) of untreated (R) and plasma-treated samples (P) at Day 7 immersion in water and ADBAC.

Table S11. Two-way ANOVA analysis Log Reduction results of W1pet, W2CEL/PET, W3cotton, and their plasma-treated (P) samples with bacteria type and material type as the factors at significant level of 0.05.

SUMMARY	**Q**	**W1RQ**	**W2RQ**	**W3RQ**	**W1PQ**	**W2PQ**	**W3PQ**	**Total**
				S. aureus				
Count	3	3	3	3	3	3	3	2.10E+01
Sum	18.5539	19.7937	19.7734	3.39208	23.6001	11.0987	3.48502	9.97E+01
Average	6.1846	6.5979	6.5911	1.13069	7.86670	3.6996	1.1617	4.75E+00
Variance	4.9116	0.4480	1.8974	0.3773	2.0971	1.4461	0.2541	7.99E+00
				E.coli				
Count	3	3	3	3	3	3	3	2.10E+01
Sum	24.5977	15.5365	12.9283	5.0664	16.0119	20.4769	8.8295	1.03E+02
Average	8.1992	5.1788	4.3095	1.6888	5.3373	6.8256	2.9432	4.93E+00
Variance	0.3644	1.1951	2.1555	0.8111	1.1691	1.4960	3.1244	5.43E+00
				Total				
Count	6	6	6	6	6	6	6	
Sum	43.1516	35.3302	32.7018	8.4585	39.6120	31.5756	12.3146	
Average	7.1919	5.8884	5.4503	1.4097	6.6020	5.2626	2.05242	
Variance	3.3280	1.2614	3.1830	0.5688	3.2259	4.1085	2.3035	
				ANOVA analysis				
Source of Variation	*SS*	*df*	*MS*	*F*	***P-value***	*F crit*		
Bacteria Type	0.3349	1	0.3349	0.2156	**0.6460**	4.1960		
Sample Type	178.9406	6	29.8234	19.1993	**9.5819E-09**	2.4453		
Interaction	46.0657	6	7.6776	4.9426	0.0015	2.4453		
Within	43.4942	28	1.5534					
Total	268.8353	41						

Table S12. ANOVA analysis of antimicrobial test result over storage time: control wipe samples at significant level 0.05.

Source of Variation		**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
ANOVA result of *S. aureus*	Sample Type	54.16	3	18.05	19.68	6.30615E-05	3.49030
	Storage Time	6.88	4	1.72	1.88	0.17950	3.25917
	Error	11.01	12	0.92			
	Total	72.06	19				
ANOVA result of *E. coli*	Sample Type	149.62	3	49.87	68.64	7.98187E-08	3.49030
	Storage Time	2.39	4	0.60	0.82	0.53575	3.25917
	Error	8.72	12	0.73			
	Total	160.72	19				

Table S13. ANOVA analysis of antimicrobial test result over storage time: plasma-treated wipe samples at significant level 0.05.

Source of Variation		**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
ANOVA result of *S. aureus*	Sample Type	74.19	3	24.73	23.57	2.55926E-05	3.49029
	Storage Time	18.45	4	4.61	4.40	0.02035	3.25917
	Error	12.59	12	1.05			
	Total	105.23	19				
ANOVA result of *E. coli*	Sample Type	156.04	3	52.01	54.09	3.03227E-07	3.49029
	Storage Time	22.15	4	5.54	5.76	0.00799	3.25917
	Error	11.54	12	0.96			
	Total	189.73	19				