

Article

# Colloidal Stability of Positively Charged Dispersions of Styrene and Acrylic Copolymers in the Presence of $\text{TiO}_2$ and $\text{CaCO}_3$

Kamil Wojciechowski \*<sup>1</sup>, Małgorzata Gutarowicz, Kamil Janke, Ilona Jurek, Marcin Kaczorowski<sup>2</sup>, Jolanta Mierzejewska<sup>2</sup> and Paweł Parzuchowski

Faculty of Chemistry, Warsaw University of Technology, Noakowskiego 3, 00-664 Warsaw, Poland; mgutarowicz@wp.pl (M.G.); jk.janke2@gmail.com (K.J.); ijurek@ch.pw.edu.pl (I.J.); mtkaczorowski@gmail.com (M.K.); jmierzejewska@ch.pw.edu.pl (J.M.); pparzuch@ch.pw.edu.pl (P.P.)

\* Correspondence: kamil.wojciechowski@ch.pw.edu.pl; Tel.: +48-22-2345106

Received: 28 December 2018; Accepted: 28 January 2019; Published: 30 January 2019



**Abstract:** Increasing antibiotic resistance of several pathogenic microorganisms calls for alternative approaches to prevent spreading of bacterial diseases. We propose to employ for this purpose coatings obtained from positively charged latex dispersions. In this contribution we characterize aqueous mixed dispersions containing  $\text{TiO}_2$  or  $\text{CaCO}_3$  and methyl methacrylate-ethyl acrylate or styrene-ethyl acrylate copolymers synthesized using a cationic surfactant, cetyltrimethylammonium bromide as an emulsifier. Particle size, electrokinetic ( $\zeta$ ) potential of the mixed dispersions and the resulting thin films, as well as antimicrobial properties of the latter are described. The  $\text{TiO}_2$  and  $\text{CaCO}_3$  dispersions were stabilised with polyethyleneimine (PEI) and optimum pH for the mixed dispersions were chosen on the basis of  $\zeta$ -potential measurements. For  $\text{TiO}_2$ , the maximum  $\zeta = +35$  mV was found at pH 7.5, and for  $\text{CaCO}_3$ , pH was set at 8.2 ( $\zeta = +38$  mV), to prevent its dissolution. In most 1:1 mixtures of  $\text{TiO}_2$  or  $\text{CaCO}_3$  with the cetyltrimethylammonium bromide (CTAB)-stabilised latex dispersions, two distinct particles populations were observed, corresponding to the bare latex and bare  $\text{TiO}_2$  or  $\text{CaCO}_3$  fractions. Films made of the mixed dispersions remained positively charged and showed antimicrobial activity similar or reduced with respect to the bare polymer films.

**Keywords:** latex; antimicrobial coatings; titania; paints; CTAB

## 1. Introduction

Bacterial infections are becoming increasingly difficult to prevent and fight because of a growing number of antibiotic-resistant pathogenic strains. Some areas, like hospitals or nurseries, are especially prone to bacterial infections spreading. One of the most effective ways to prevent them is to apply antibacterial coatings, e.g., in the form of wall paints. Currently, most of the antibacterial coatings of this type rely on antibiotics, triclosan, chlorhexidine, quaternary ammonium compounds or metals (Cu, Ag, Zn) [1].

Natural defence systems of all higher organisms rely on both specific and non-specific mechanisms. While the former involves very complex cascades of biochemical reactions, the non-specific defence system employs relatively simple oligopeptides circulating in the body and available well before the specific immune response is deployed by the organism under attack. The antimicrobial peptides (AMP) are low-molecular weight oligomers accommodating positive charge and a stiff hydrophobic fragment [2]. The two features allow them to bind to negatively charged walls and cytoplasmic membranes of any bacteria. The consequent alteration of the bilayer structure of the membrane may kill both Gram-negative and Gram-positive bacteria. AMP typically consist of 15–20 amino acids with a net

charge of at least +2 [3]. Three types of AMP structures,  $\alpha$ -helical cathelicidins,  $\beta$ -sheet defensins and kinocidins, dominate in the human defence system [4]. Several types of AMP-mimetic molecules have been synthesized recently [5,6], e.g., phenylene ethynylene oligomers and polymers [7,8], arylamide foldamers [9], arginine-based cationic surfactants [10], aryl-based alkoxyamines [11] or simple quaternary ammonium salts [12–16]. The latter, e.g., cetyltrimethylammonium or tetraethylammonium bromides and cetylpyridinium or benzalkonium chloride, are extensively used in antibacterial and antifungal formulations. Oxyethylated quaternary ammonium salts with C<sub>8</sub> to C<sub>16</sub> alkyl chains have been introduced into antimicrobial paints based on acrylic polymers at 1 wt % (with respect to the polymer solids). The resulting coatings provided significant activity against several Gram-positive and Gram-negative bacteria, as well as fungi and viruses [17]. The quaternary ammonium salts have also been successfully deposited onto ethylene-vinyl acetate latex in a form of nonstoichiometric complex with polyacrylic acid [18].

Positively charged latexes have been known in the literature for many years. For example, Goodwin et al. reported on synthesis of cationic polystyrene particles in the absence of an added surfactant, with the positive charge impaired solely by the use of cationic initiators [19]. Antonietti et al. employed different combinations of anionic (sodium dodecyl sulfate) and cationic (cetyltrimethylammonium chloride or dodecyltrimethylammonium bromide) surfactants to control the size of polystyrene particles [20]. Nevertheless, most previous studies on cationic polymers obtained with cetyltrimethylammonium bromide (CTAB) and similar tetraalkylammonium salts focused mainly on synthesis and characterisation of the particles [21,22]. The group of Carmona-Ribeiro studied solvent-cast films obtained by spin-coating chloroformic solutions of quaternary ammonium salts with polystyrene and poly(methyl methacrylate). The hybrid films showed antimicrobial activity, especially against *S. aureus* and *E. coli* [23–26]. To the best of our knowledge, however, no study was so far devoted to colloidal stability in the mixed system containing positively charged latex and inorganic particles in the context of paint formulations. “Classical” paints are based on negatively charged polymeric binders and fillers/pigments stabilised at high pH with water-soluble polymers, e.g., polyacrylates. Reversing the sign of the film-forming latex requires a concomitant reversal of the surface charge of other components, especially the fillers/pigments, to prevent their aggregation due to electrostatic attraction. While some reports exist on synthesis and characterisation of the positively charged latex particles, their films, even in simple systems without inorganic particles, have not been extensively studied so far [27].

In our recent contribution we have described synthesis and characterisation of positively charged methyl methacrylate-ethyl acrylate and styrene-ethyl acrylate copolymers latexes. The positive charge was introduced by employing a cationic surfactant, cetyltrimethylammonium bromide (CTAB) as an emulsifier. The surface charge brought by CTAB was maintained even after extensive dialysis and provided antibacterial activity against Gram-negative (*E. coli*), Gram-positive (*S. aureus*, *B. subtilis*) bacteria and yeast (*C. albicans*) [28]. In the present contribution we describe an extension of the previous study, by analysing compatibility of the positively charged latexes with two model inorganic particles used as fillers and pigments in real water-borne paints: CaCO<sub>3</sub> and TiO<sub>2</sub>. The former represents a typical filler used extensively in commercial paint formulations, mainly for its very competitive price. The refractive index of CaCO<sub>3</sub> is, however, relatively close to that of air ( $n = 1.7$ ), thus in order to achieve better optical parameters of a paint, TiO<sub>2</sub>, with  $n = 2.6$ , is often added as a pigment to white paints. The main aim of the present study is to verify if colloidal stability and antimicrobial properties of the positively charged latexes could be preserved in the presence of the mineral particles. This will be achieved by preventing heteroaggregation between the small latex particles (of the order of 100 nm) and larger TiO<sub>2</sub> and CaCO<sub>3</sub> particles (of the order of 1  $\mu$ m).

## 2. Materials and Methods

The synthesis and characterisation of methyl methacrylate-ethyl acrylate and styrene-ethyl acrylate copolymers were described in detail in our previous paper [28]. Briefly, all polymers were

obtained in an emulsion polymerization process using a cationic surfactant, cetyltrimethylammonium bromide (CTAB) as an emulsifier and either sodium persulphate,  $\text{Na}_2\text{S}_2\text{O}_8$  or (2,2'-azobis (2-methylpropionamide) dichloride, AAPH, as an initiator (referred to as "anionic" and "cationic", respectively). For comparison, a methyl methacrylate-ethyl acrylate co-polymer was also prepared using sodium dodecyl sulphate (SDS) as an emulsifier and the "anionic" initiator, in order to obtain a negatively charged latex. The reaction mixtures containing 30% dry mass of polymer solids were dialyzed against Milli-Q water (Millipore, Merck KGaA, Darmstadt, Germany) for 3 days. The efficiency of dialysis was verified by monitoring the electrical conductivity.  $\text{TiO}_2$  and  $\text{CaCO}_3$  nanopowders with  $21 \pm 10$  nm and ca. 90 nm diameter, respectively, were purchased from PlasmaChem GmbH (cat. no. PL-TiO-P25 and PL-CACOU). Polyethylenimine (PEI), average  $M_w \approx 25000$  by LS was purchased from Sigma-Aldrich (cat. no. 408727, Poznań, Poland). Milli-Q water (Millipore, Merck KGaA, Darmstadt, Germany) was used for all experiments. The nanopowders were dispersed in water at 30 g/L using Ultraturrax T25 (10,000 rpm, 15 min, IKA<sup>®</sup>-Werke GmbH & Co. KG, Staufen, Germany) in the absence or presence of PEI (1% with respect to the polymer solids). pH of the latex and inorganic dispersions was regulated using NaOH and HCl.

### 2.1. Size and $\zeta$ -Potential Measurements

Particle size and  $\zeta$ -potentials of latex dispersion were measured at 25 °C using a Malvern Zetasizer 3000HS (Malvern Instruments Ltd., Malvern, UK) after a 1000-fold dilution (0.03% dry mass of polymer solids). In calculations of  $\zeta$ -potential, the classical Helmholtz–Smoluchowski formula was used. Particle sizes are presented as the Sauter mean diameter,  $D_{32}$ , defined as follows:

$$D_{32} = \frac{\sum N_i \times d_i^3}{\sum N_i \times d_i^2} \quad (1)$$

where:  $N_i$  is a percentage of the total scattering intensity for a given scattering droplet diameter ( $d_i$ /nm).

$\zeta$ -potential measurements for the films prepared from mixed polymeric-inorganic dispersions were performed using a SurPASS Electrokinetic Analyzer equipped with an adjustable gap cell from Anton Paar GmbH (Graz, Austria) by measuring a pressure—streaming current dependency in NaBr solution ( $10^{-3}$  M). For each measurement two pieces of a given polymer film cast on a foil (20 mm × 10 mm) were precisely cut and installed in an adjustable gap cell of the SurPASS instrument (Anton Paar, Graz, Austria), separated with a 100  $\mu\text{m}$ -thick spacer. The foil used as a support was made of poly(ethylene terephthalate) (PET). More details can be found in ref. [28]. The films were spread using a metal bar with 120  $\mu\text{m}$  gap and left to evaporate for 24 h.

### 2.2. Antimicrobial Testing

The following bacteria: *Escherichia coli* ATCC8739, *Staphylococcus aureus* ATCC6538, *Bacillus subtilis* ATCC6633, *Pseudomonas aeruginosa* ATCC9027 and yeast *Candida albicans* ATCC10231 were applied in antimicrobial activity tests. Bacteria were grown in MHB medium (Mueller-Hinton broth, Biocorp, Warsaw, Poland) while yeast in SAB medium (Sabouraud broth, Merck KGaA, Darmstadt, Germany) overnight (about 18 h) at  $36 \text{ °C} \pm 1 \text{ °C}$  with shaking at 240 rpm (Benchtop shaker SI-600R, LabCompanion, Jeiotech, Daejeon, Korea). Subsequently, the overnight cultures were diluted in fresh media to obtain the required concentration of colony forming units per mL ( $\text{CFU} \cdot \text{mL}^{-1}$ ). Antimicrobial tests were performed using the "dry film method" as described in our previous paper [28]. First, the 100  $\mu\text{L}$  portions of each polymer were spotted on the bottom of a sterile Petri dish (Bionovo, Legnica, Poland) and left at room temperature to dry for three days. Then, half of the dried polymer spots placed on the bottom of a Petri dish were washed with a 20 mL portion of sterile Milli-Q water for 15 min at room temperature. After that, the MHA medium (Mueller-Hinton agar, Merck, Germany) inoculated with bacteria ( $10^7 \text{ CFU} \cdot \text{mL}^{-1}$ ) or SAB with 2% ( $w/v$ ) agar inoculated with yeast

( $10^6$  CFU·mL<sup>-1</sup>) were poured on the dried polymers. When the media solidified, the Petri dishes were incubated for 20 h at  $36 \text{ }^\circ\text{C} \pm 1 \text{ }^\circ\text{C}$ . Thereafter, the growth inhibition zones in solid cultures were visible if the tested polymer was toxic for microbial cells.

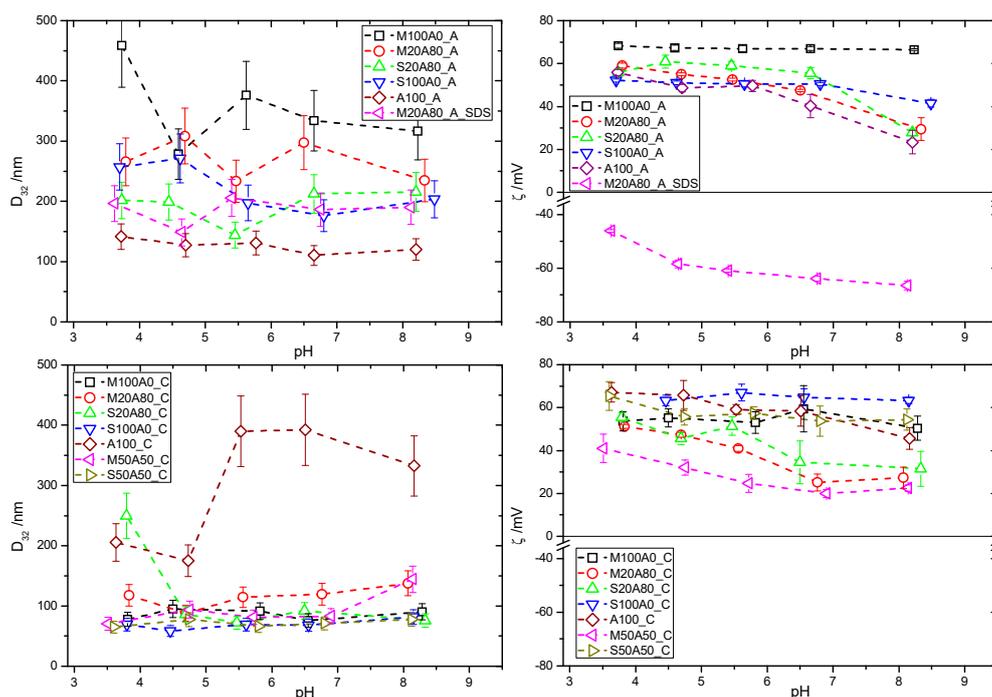
### 3. Results

The synthesis and characterisation of the styrene-acrylic and methacrylic-acrylic copolymers was described in our previous paper [28]. The composition of the copolymers chosen for the present study, determined from the <sup>1</sup>H-NMR measurements is shown in Table 1.

**Table 1.** Composition of co-polymers employed in the present study (monomer content from <sup>1</sup>H-NMR measurements described in [28]).

Sample	Monomer Content (mol%)			Surfactant	Initiator	Yield (%)
	ST	MMA	EA			
M100A0_A	0.0	100.0	0.0	CTAB	Na <sub>2</sub> S <sub>2</sub> O <sub>8</sub>	84.6
S100A0_A	100.0	0.0	0.0	CTAB	Na <sub>2</sub> S <sub>2</sub> O <sub>8</sub>	75.4
M20A80_A	0	21.5	78.5	CTAB	Na <sub>2</sub> S <sub>2</sub> O <sub>8</sub>	60.1
S20A80_A	24.5	0.0	75.5	CTAB	Na <sub>2</sub> S <sub>2</sub> O <sub>8</sub>	96.7
A100_A	0	0.0	100.0	CTAB	Na <sub>2</sub> S <sub>2</sub> O <sub>8</sub>	75.7
M20A80_A_SDS	0	23.1	76.9	SDS	Na <sub>2</sub> S <sub>2</sub> O <sub>8</sub>	87.6
M100A0_C	0	100.0	0.0	CTAB	AAPH	96.8
S100A0_C	100.0	0.0	0.0	CTAB	AAPH	96.8
M20A80_C	0	19.6	80.4	CTAB	AAPH	97.1
S20A80_C	20.9	0.0	79.1	CTAB	AAPH	99.9
M50A50_C	0	44.8	55.2	CTAB	AAPH	93.0
S50A50_C	54.5	0.0	45.5	CTAB	AAPH	92.4
A100_C	0	0.0	100.0	CTAB	AAPH	94.0

In the first part of the study, the effect of pH on colloidal stability of individual dispersions of latex and mineral particles was analysed. The latex dispersions were dialysed in order to remove excess CTAB and unreacted monomers and then diluted with Milli-Q water by a factor of  $10^3$  for the subsequent measurements of pH dependence of the size and  $\zeta$ -potential (Figure 1). The Sauter diameter,  $D_{32}$  of all co-polymers showed a rather weak pH dependence in the pH range 3.0–8.5 for all polymers, suggesting their high colloidal stability under the employed experimental conditions. As already noted in our previous study at neutral pH, almost all latex particles obtained with the cationic initiator, AAPH, are 2–3 times smaller than their analogues obtained using the anionic initiator, sodium persulfate. The only exception is the A\_100\_C and A\_100\_A couple: the cationic and anionic poly(ethyl acrylate). The  $\zeta$ -potential measurements confirmed that surface charge of all latexes obtained in presence of CTAB remains positive up to at least pH 8.5. This weak pH dependency is not surprising in view of the fact that the majority of surface charge originates from the presence of the quaternary ammonium cations of CTAB molecules trapped within and adsorbed on the surface of the latex particles. The nature of the initiator used in the synthesis does not play any significant role in determining the  $\zeta$ (pH) dependency. In the case of analogous co-polymers obtained in presence of an anionic surfactant–sodium dodecyl sulphate (SDS), surface potential is negative, as expected.



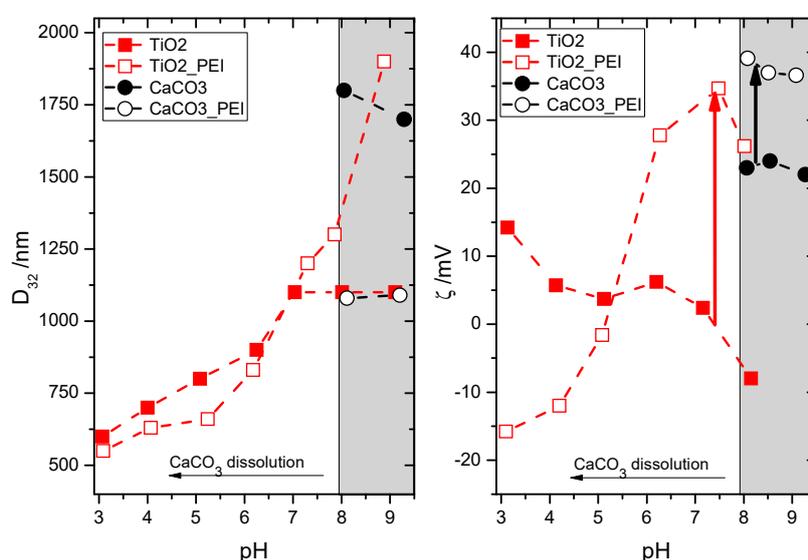
**Figure 1.** Sauter diameter,  $D_{32}$  (left) and  $\zeta$ -potential (right) for the polymer dispersions with anionic (top row) and cationic (bottom row) initiator (see Table 1 for compositions).

The wide pH window of colloidal stability of all cationic latex particles renders them good potential candidates for mixing with mineral particles constituting the second major component of most water borne paints—pigments and fillers. In classical paints, because of high pH and the presence of anionic polyelectrolytes (e.g., polyacrylic acid) used as stabilisers, the mineral particles (e.g.,  $\text{TiO}_2$  or  $\text{CaCO}_3$ ) are negatively charged. This provides an electrostatic barrier against their coalescence with the negatively charged latex. In the present case of positively charged film-forming co-polymers, the mineral particles should preferably also be positively charged to hinder their heteroaggregation with the latex. In the case of mineral particles, the surface groups can be protonated and deprotonated depending on pH and presence of additional stabilisers. The experimentally determined pH dependencies of Sauter diameter ( $D_{32}$ ) and  $\zeta$ -potential for both types of model mineral particles are shown in Figure 2 as filled points. For  $\text{TiO}_2$ , decreasing pH helps to partially disperse the particle aggregates. At pH 3, the size can be reduced to  $D_{32} = 600$  nm using the applied homogenization procedure, although it should be stressed that this value is still much higher than the primary size of the particles claimed by the supplier ( $21 \pm 10$  nm). Electrokinetic measurements confirm that decreasing pH promotes protonation of  $\text{TiO}_2$  and at pH 3,  $\zeta = +14$  mV. As expected, increasing pH reduces the positive surface charge, which favours aggregation manifested in increase of  $D_{32}$ . For  $\text{CaCO}_3$ , dissolution in neutral and acidic media limits the pH range of colloidal stability. Nevertheless, even above pH 8 the surface of the employed  $\text{CaCO}_3$  nanopowder is positively charged ( $\zeta > +20$  mV), although the aggregate size remains much above the primary particle limit (1800 nm vs ca. 90 nm).

Both  $\text{TiO}_2$  (at pH 3) and  $\text{CaCO}_3$  (at pH > 8) without additional stabilisation are only moderately positively charged. Thus, an effort to increase their  $\zeta$ -potential was taken by adding a cationic polyelectrolyte, in analogy to the anionic one (poly(acrylic acid)) in “classical” paint formulations containing negatively charged latexes. The addition of 1% polyethyleneimine (PEI) does not change the size(pH) dependency for  $\text{TiO}_2$  up to pH 7. Surprisingly, above this value the aggregate size increases, despite the reversal of the  $\zeta$ (pH) dependency. Apparently, increasing pH strengthens the attractive interactions between PEI-covered  $\text{TiO}_2$  particles. Under these conditions, the adsorbed PEI chains might be heterogeneously protonated, which would result in patches of more and less protonated polymer fragments at different locations of the particle surface. These patches might

promote aggregation between the neighbouring PEI-covered particles because of reduced electrostatic repulsion. Consequently, the optimum conditions to prevent aggregation between PEI-covered particles was found at pH 7.5, where  $\zeta$ -potential is more positive by over 30 mV than in the absence of PEI. In the case of  $\text{CaCO}_3$ , addition of PEI also increased the  $\zeta$ -potential values within the stability window (pH 8–9) by about 15 mV. As expected, the increase of surface charge helped to reduce the aggregate size of  $\text{CaCO}_3$  down to about 1100 nm.

Based on the pH dependencies for both latex (Figure 1) and mineral (Figure 2) particles, the optimum conditions to test their compatibility are selected as pH 7.5 for  $\text{TiO}_2$  and 8.2 for  $\text{CaCO}_3$ , both stabilised with PEI (1% with respect to the polymer solids). Under these conditions,  $\zeta$ -potential for both particles exceeds +30 mV, which should allow for effective electrostatic stabilisation. For the latex particles, the surface charge at elevated pH is reduced with respect to the acidic and neutral conditions, but still remains positive ( $\zeta > +20$  mV). For some polymers (M100A0 and S100A0), the values reaching +40 or even +60 mV are observed at pH > 8.0, but unfortunately their high rigidity (related to the absence of ethyl acrylate monomers) prevents films formation. In view of the potential applications in antimicrobial coatings, they will not be included in the subsequent compatibility study.



**Figure 2.** Sauter diameter,  $D_{32}$  (left) and  $\zeta$ -potential (right) for the  $\text{TiO}_2$  (red squares) and  $\text{CaCO}_3$  (black circles) dispersions in the absence (closed symbols) and presence of 1% PEI (open symbols). Grey rectangle at high pH corresponds to the region of  $\text{CaCO}_3$  stability against dissolution.

In order to test the compatibility of the positively charged latexes and the mineral particles, both dispersions were mixed in 1:1 ratio (dry weight basis), which corresponds to a typical ratio of polymer to fillers/pigments in water-borne paint formulations. The inorganic nanoparticles were first dispersed in aqueous PEI solution at fixed pH (7.5 for  $\text{TiO}_2$  and 8.2 for  $\text{CaCO}_3$ ) and mixed with the following polymer dispersions at the same pH: M20A80\_A, S20A80\_A, M20A80\_C, S20A80\_C, A100\_A, A100\_C and M20A80\_A\_SDS. The mixtures were left to equilibrate for two days prior to measuring their size and  $\zeta$ -potential (Table 2). In both mixtures containing the SDS-stabilised polymer dispersion (M20A80\_A\_SDS) a precipitate was formed at the bottom, pointing to extensive heteroaggregation with  $\text{CaCO}_3$  and  $\text{TiO}_2$ . In the majority of remaining mixtures two distinct fractions of particles could be observed, one corresponding to the size of latex particles, and one to the size of individual  $\text{TiO}_2$  or  $\text{CaCO}_3$  aggregates. For both methacrylate-based latexes (M20A80\_A and M20A80\_C), only one peak corresponding roughly to the size of the mineral particles can be observed. Although the differences are less pronounced than in the pure latex particle dispersions, when mixed with  $\text{TiO}_2$  or  $\text{CaCO}_3$  the anionic latexes are on average smaller than the cationic ones. As in the case of the bare latex dispersions, size distributions of the mixtures containing pure poly(acrylate) polymers are also distinct.

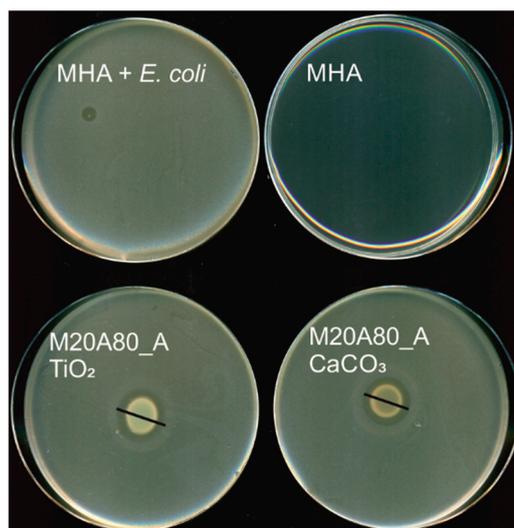
The latex-CaCO<sub>3</sub> mixtures show very similar and high values of  $\zeta$ -potential ( $38 \pm 2$  mV) independently of the monomer composition and the initiator used. These values are very close to that observed in bare CaCO<sub>3</sub>. On the other hand, for the latex-TiO<sub>2</sub> mixtures, a significant reduction of  $\zeta$ -potential could be noticed ( $14 \pm 3$  mV), with the exception of pure poly(acrylate), where even a slight increase is observed.

**Table 2.** Size and  $\zeta$ -potential values for the mixed dispersions containing cetyltrimethylammonium bromide (CTAB)-stabilised latexes and TiO<sub>2</sub> or CaCO<sub>3</sub> nanopowders dispersed in 1% PEI (with respect to the polymer solids) at pH 7.5 (TiO<sub>2</sub>) and 8.2 (CaCO<sub>3</sub>).

Mineral Particles	CaCO <sub>3</sub> + 1% PEI pH 8.2		TiO <sub>2</sub> + 1% PEI pH 7.5		
	Polymer	Size (nm)	$\zeta$ (Mv)	Size (nm)	$\zeta$ (Mv)
M20A80_A		190 $\pm$ 28; 708 $\pm$ 106	+35 $\pm$ 4	503 $\pm$ 45	+9 $\pm$ 1
S20A80_A		126 $\pm$ 19; 495 $\pm$ 74	+37 $\pm$ 4	92 $\pm$ 15; 792 $\pm$ 114	+16 $\pm$ 2
M20A80_C		108 $\pm$ 16; 754 $\pm$ 113	+39 $\pm$ 4	767 $\pm$ 113	+12 $\pm$ 2
S20A80_C		100 $\pm$ 15; 667 $\pm$ 100	+39 $\pm$ 4	84 $\pm$ 12; 463 $\pm$ 70; 917 $\pm$ 140	+19 $\pm$ 2
A100_A		146 $\pm$ 22; 1130 $\pm$ 170	+36 $\pm$ 4	460 $\pm$ 43	+31 $\pm$ 4
A100_C		75 $\pm$ 11; 170 $\pm$ 26	+39 $\pm$ 4	370 $\pm$ 48; 1280 $\pm$ 185	+42 $\pm$ 4

To test the electrokinetic and antimicrobial performance of the cationic polymer-TiO<sub>2</sub> and cationic polymer-CaCO<sub>3</sub> mixed films, two polymers were selected from the set described above: M20A80\_A and S20A80\_A. They were chosen based on our previous study of pure polymeric films [28], where both showed good mechanical and antimicrobial properties. For the electrokinetic tests, thin films containing the polymer (M20A80\_A or S20A80\_A) and mineral particles (TiO<sub>2</sub> or CaCO<sub>3</sub>) in 1:1 ratio (dry solids basis) were cast on a PET foil and fitted into the measuring cell of SurPass analyser.  $\zeta$ -potential values were calculated from the streaming current measurements recorded by pushing an aqueous electrolyte ( $10^{-3}$  M NaBr) through a thin channel formed by the studied thin films supported on two parallel PET foils. The films containing TiO<sub>2</sub> showed similar  $\zeta$ -potential values of ( $+24 \pm 1$  mV for M20A80\_A) and ( $+25 \pm 1$  mV for S20A80\_A), which is close to the values for the bare polymers. Unexpectedly, for CaCO<sub>3</sub>, the  $\zeta$ -potential values obtained for the films largely exceeded those measured for the starting dispersions:  $+82 \pm 1$  mV for M20A80\_A) and ( $+71 \pm 6$  mV for S20A80\_A).

For antimicrobial tests, the mixed polymer-inorganic films were cast at the bottom of a sterile Petri dish using a pipette (100  $\mu$ L) and left to dry for 72 h. Half of the spots were rinsed with 20 mL of Milli-Q water in order to rinse off possible water-soluble components (not adsorbed CTAB, PEI, unreacted monomers and/or initiator) [29–32]. The dried polymer-inorganic films were then covered with the solid media inoculated with the bacteria (*P. aeruginosa*, *E. coli*, *S. aureus*) and fungi (*C. albicans*). The microbial growth was observed as transparent zones above and around the films. The representative photographs of antimicrobial tests in solid culture media are presented in Figure 3 and the quantitative results are collected in Table 3. The data without TiO<sub>2</sub> and CaCO<sub>3</sub> from our previous paper [28] are also shown for comparison. In general, the methacrylate derivative (M20A80\_A) showed much broader antimicrobial activity than the styrene analogue (S20A80\_A). The mixed films of the former show practically the same activity against *E. coli* as the bare polymer film, although the activity ceases after rinsing with Milli-Q, similar to the case without the mineral particles. Only one composition (M20A80\_A with TiO<sub>2</sub>) showed some activity against *P. aeruginosa*, but again it disappeared after rinsing with water. For *S. aureus*, the presence of mineral particles reduces the bacterial growth inhibition zones with respect to the bare M20A80\_A and S20A80\_A films, although for M20A80\_A with TiO<sub>2</sub>, even after rinsing some antibacterial activity remained. The best results, however, were obtained against the yeast *C. albicans*, where the antifungal activity of the M20A80\_A films containing both CaCO<sub>3</sub> and TiO<sub>2</sub> remained practically unchanged even after rinsing.



**Figure 3.** Representative photographs of antimicrobial tests (against *E. coli*) in solid culture media. Mueller-Hinton agar (MHA): control plate, MHA + *E. coli*: control plate inoculated with *E. coli*. The black lines indicate the diameters of the zone of growth inhibition.

**Table 3.** Bacterial and yeast susceptibility tests in solid culture media. The latexes were spotted on the bottom of empty Petri dish and left to dry, and subsequently covered by solid cultures of microorganisms. The mean ( $\pm$ SD,  $n = 3$ ) diameters of the zones of growth inhibition are expressed in (mm); nt: not tested.

Polymer	Additive	<i>P. aeruginosa</i>		<i>E. coli</i>		<i>S. aureus</i>		<i>C. albicans</i>	
		Not Rinsed	Rinsed with Milli-Q	Not Rinsed	Rinsed with Milli-Q	Not Rinsed	Rinsed with Milli-Q	Not Rinsed	Rinsed with Milli-Q
M20A80_A	-	0	nt	18 $\pm$ 6	0	82 $\pm$ 4	30 $\pm$ 5	14 $\pm$ 1	0
	CaCO <sub>3</sub>	0	0	16 $\pm$ 1	0	21 $\pm$ 3	0	16 $\pm$ 1	14 $\pm$ 1
	TiO <sub>2</sub>	11 $\pm$ 1	0	18 $\pm$ 1	0	22 $\pm$ 4	10 $\pm$ 2	16 $\pm$ 1	13 $\pm$ 1
S20A80_A	-	0	nt	0	nt	30 $\pm$ 4	nt	13 $\pm$ 2	nt
	CaCO <sub>3</sub>	0	0	0	0	15 $\pm$ 1	0	0	0
	TiO <sub>2</sub>	0	0	0	0	14 $\pm$ 2	0	0	0

#### 4. Discussion

Mixed dispersions containing polymer and mineral particles must be stabilised against both homo- and heteroaggregation. According to the Derjaguin–Landau–Verwey–Overbeek (DLVO) theory, the total interaction potential between two particles depends on an interplay between electrostatic and van der Waals interactions [33]. In the case of homoaggregates, formed between the latex or between the mineral particles, the Hamaker constant,  $H$ , characterizing the van der Waals contribution, is always positive, leading to attractive interactions between like particles. The latter is especially high for TiO<sub>2</sub> ( $H = 5.35 \times 10^{-20}$  J) [34], although this value is reduced in most commercially available titania nanopowders by the presence of inorganic coatings (e.g., alumina, with  $H = 2.75 \times 10^{-20}$  J) [35] or surface-active stabilisers (e.g., PEI, like in the present case). PEI, in addition to lowering the van der Waals attraction potential, provides positive surface charge manifesting in positive  $\zeta$ -potential and promoting repulsive electrostatic interactions. Nevertheless, addition of 1% PEI was not sufficient in the present case to fully disperse the secondary aggregates of TiO<sub>2</sub>. Also, for CaCO<sub>3</sub>, with Hamaker constant,  $H = 1.44 \times 10^{-20}$  J) [34], the effect of PEI was not sufficient to fully disperse the aggregates, despite  $\zeta > +35$  mV. On the other hand, a much smaller van der Waals attraction between polymer particles (e.g., for carboxyl-terminated polystyrene latex particles,  $H = 0.2 \times 10^{-20}$  J [36] combined with their high positive surface charge ( $+20$  mV  $< \zeta < +67$  mV, see Figure 1) resulted in very stable latex dispersions with  $D_{32}$  not showing significant changes in the studied pH range.

In the present case the van der Waals forces between latex and TiO<sub>2</sub> or CaCO<sub>3</sub> in water are most likely positive and favour their heteroaggregation. The only way to prevent the latter is the maximisation of the repulsive electrostatic interactions between the latex and the TiO<sub>2</sub>/CaCO<sub>3</sub> mineral particles. To this aim, pH of the positively charged mixed dispersions tested in the present study were chosen so that the  $\zeta$ -potentials of individual dispersions were the highest. Not surprisingly, for the mixture with the SDS-stabilised polymer (M20A80\_A\_SDS), electrostatic attraction favours heteroaggregation, which resulted in macroscopically observable precipitation. On the other hand, in almost all combinations of CTAB-stabilised latex with CaCO<sub>3</sub> (except for A\_100\_C), the presence of two distinct particle populations with sizes roughly corresponding to the individual components, and the high  $\zeta$ -potential values, confirm that heteroaggregation can indeed be prevented. Even though for TiO<sub>2</sub> the two peak populations are less distinctly pronounced and the  $\zeta$ -potential values are lower, the lack of macroscopic precipitation suggests that titania does not aggregate with the latexes under the chosen conditions (pH 7.5).

Bare polymeric films made of pure CTAB-stabilised latex dispersions showed moderately positive surface potential values in electrokinetic analysis using streaming potential/current measurements [28]. For the tested set of polymer-inorganic combinations, the nature of the polymer did not affect the electrokinetic characteristics of the mixed films. This is not surprising given the similar  $\zeta$ -potential values for both bare polymers (M20A80\_A and S20A80\_A) in the pH range 7.5–8.2 (Figure 1). The presence of TiO<sub>2</sub> in the mixed films did not change significantly  $\zeta$ -potential, in full analogy to the situation in the corresponding mixed dispersions. Unexpectedly, however, both polymers mixed with CaCO<sub>3</sub> particles produced highly positively charged films, with  $\zeta$ -potential higher by ca. 34–44 mV than the corresponding mixed dispersions. Nevertheless, these high values did not translate into increased antimicrobial activity of the films. The TiO<sub>2</sub>-doped films were in general more efficient than their more positively charged CaCO<sub>3</sub>-doped counterparts. On the other hand, despite similar electrokinetic characteristics of M20A80\_A and S20A80\_A, their bare and mineral-doped films showed clearly different antimicrobial behaviour. Most of the mixed films were less active (except for M20A80\_A against *E. coli*) and more susceptible to the activity loss upon rinsing, than their bare polymer analogues. Only antifungal activity of M20A80\_A films was improved in presence of both mineral particles—they gained resistance to rinsing with water.

## 5. Conclusions

In the present contribution we investigated colloidal stability and antimicrobial activity of mixed polymeric-mineral dispersions with positive charge brought by a cationic surfactant (cetyltrimethylammonium bromide, CTAB) and a cationic polymer (polyethyleneimine, PEI). The colloidal stability was analysed at pH 8.2 for CaCO<sub>3</sub> and 7.5 for TiO<sub>2</sub>, chosen on the basis of pH dependency of the size and  $\zeta$ -potential of the individual (polymeric and mineral) particles. The high positive  $\zeta$ -potential values for both latex and mineral dispersions at slightly basic conditions prevented their heteroaggregation and allowed to obtain mixed dispersions with separated latex and mineral particle populations. In the absence of electrostatic repulsion, the small latex particles could adsorb on the larger mineral ones, promoting their heteraggregation and eventually leading to macroscopic aggregate formation, as in the case of the negatively charged latex mixed with positively charged mineral particles. The films obtained from the mixed dispersions of CaCO<sub>3</sub> and TiO<sub>2</sub> with M20A80\_A and S20A80\_A latexes showed positive  $\zeta$ -potential values, for CaCO<sub>3</sub> reaching up to +82 mV. However, their antimicrobial activity was not improved with respect to their bare-polymer counterparts. Most mixed films lose their activity after rinsing with water, although the presence of both CaCO<sub>3</sub> and TiO<sub>2</sub> helped to maintain antifungal activity of M20A80\_A against *C. albicans*. The mode of action of quaternary ammonium salts relies on either contact killing or releasing the antimicrobial agents into the neighbourhood. A recent study of Melo et al. [25] showed that CTAB, employed in the present system, is more likely to operate by leaching out from the film to exert its antimicrobial activity. No clear correlation between the magnitude of  $\zeta$ -potential and antimicrobial activity/selectivity could

be derived from the present results. This suggests that the mechanism of antimicrobial activity of the mixed polymer-mineral films is more complex than simple electrostatic interaction.

**Author Contributions:** Conceptualization, K.W.; methodology, K.W., J.M., P.P.; investigation, I.J., M.K., M.G., K.J.; data curation, J.M., K.J., K.W.; writing, K.W.; funding acquisition, K.W.

**Funding:** This research (including scholarships for I.J. and M.K.) was funded by The National Science Centre (Poland), project “Antimicrobial Polymer Films” (UMO-2015/19/B/ST4/01748) and by Warsaw University of Technology.

**Acknowledgments:** Agnieszka Borkowska, Marcin Mrowiński and Dominik Gutowski are acknowledged for technical assistance.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Cloutier, M.; Mantovani, D.; Rosei, F. Antibacterial Coatings: Challenges, Perspectives, and Opportunities. *Trends Biotechnol.* **2015**, *33*, 637–652. [[CrossRef](#)] [[PubMed](#)]
2. Shaik, S. Is my chemical universe localized or delocalized? is there a future for chemical concepts? *New J. Chem.* **2007**, *31*, 2015. [[CrossRef](#)]
3. Findlay, B.; Zhanel, G.G.; Schweizer, F. Cationic amphiphiles, a new generation of antimicrobials inspired by the natural antimicrobial peptide scaffold. *Antimicrob. Agents Chemother.* **2010**, *54*, 4049–4058. [[CrossRef](#)] [[PubMed](#)]
4. Koprivnjak, T.; Peschel, A. Bacterial resistance mechanisms against host defense peptides. *Cell. Mol. Life Sci.* **2011**, *68*, 2243–2254. [[CrossRef](#)]
5. Colomer, A.; Pinazo, A.; García, M.T.; Mitjans, M.; Vinardell, M.P.; Infante, M.R.; Martínez, V.; Pérez, L. PH-sensitive surfactants from lysine: Assessment of their cytotoxicity and environmental behavior. *Langmuir* **2012**, *28*, 5900–5912. [[CrossRef](#)] [[PubMed](#)]
6. Salay, L.C.; Ferreira, M.; Oliveira, O.N.; Nakaie, C.R.; Schreier, S. Headgroup specificity for the interaction of the antimicrobial peptide tritriptin with phospholipid Langmuir monolayers. *Colloids Surf. B Biointerfaces* **2012**, *100*, 95–102. [[CrossRef](#)] [[PubMed](#)]
7. Wang, Y.; Chi, E.Y.; Schanze, K.S.; Whitten, D.G. Membrane activity of antimicrobial phenylene ethynylene based polymers and oligomers. *Soft Matter* **2012**, *8*, 8547. [[CrossRef](#)]
8. Yang, L.; Gordon, V.D.; Mishra, A.; Som, A.; Purdy, K.R.; Davis, M.A.; Tew, G.N.; Wong, G.C.L. Synthetic antimicrobial oligomers induce a composition-dependent topological transition in membranes. *J. Am. Chem. Soc.* **2007**, *129*, 12141–12147. [[CrossRef](#)]
9. Mensa, B.; Kim, Y.H.; Choi, S.; Scott, R.; Caputo, G.A.; DeGrado, W.F. Antibacterial mechanism of action of arylamide foldamers. *Antimicrob. Agents Chemother.* **2011**, *55*, 5043–5053. [[CrossRef](#)]
10. Castillo, J.; Pinazo, A.; Carilla, J.; Infante, M.R.; Alsina, M.A.; Haro, I.; Clapés, P. Interaction of antimicrobial arginine-based cationic surfactants with liposomes and lipid monolayers. *Langmuir* **2004**, *20*, 3379–3387. [[CrossRef](#)]
11. Thaker, H.D.; Som, A.; Ayaz, F.; Lui, D.; Pan, W.; Scott, R.W.; Anguita, J.; Tew, G.N. Synthetic mimics of antimicrobial peptides with immunomodulatory responses. *J. Am. Chem. Soc.* **2012**, *134*, 11088–11091. [[CrossRef](#)] [[PubMed](#)]
12. Yu, Q.; Zhang, B.; Ma, F.; Jia, C.; Xiao, C.; Zhang, B.; Xing, L.; Li, M. Novel mechanisms of surfactants against *Candida albicans* growth and morphogenesis. *Chem. Biol. Interact.* **2015**, *227*, 1–6. [[CrossRef](#)] [[PubMed](#)]
13. Vieira, D.B.; Carmona-Ribeiro, A.M. Cationic lipids and surfactants as antifungal agents: Mode of action. *J. Antimicrob. Chemother.* **2006**, *58*, 760–767. [[CrossRef](#)] [[PubMed](#)]
14. Ishikawa, S.; Matsumura, Y.; Katoh-Kubo, K.; Tsuchido, T. Antibacterial activity of surfactants against *Escherichia coli* cells is influenced by carbon source and anaerobiosis. *J. Appl. Microbiol.* **2002**, *93*, 302–309. [[CrossRef](#)] [[PubMed](#)]
15. Montazer, M.; Rangchi, F.; Siavoshi, F. Preparation of Protective Disposable Hygiene Fabrics for Medical Applications. In *Medical and Healthcare Textiles*; Elsevier: New York, NY, USA, 2010; pp. 164–170, ISBN 9781845692247.

16. Venkata Nancharaiah, Y.; Reddy, G.K.K.; Lalithamasana, P.; Venugopalan, V.P. The ionic liquid 1-alkyl-3-methylimidazolium demonstrates comparable antimicrobial and antibiofilm behavior to a cationic surfactant. *Biofouling* **2012**, *28*, 1141–1149. [[CrossRef](#)] [[PubMed](#)]
17. Fulmer, P.A.; Wynne, J.H. Development of broad-spectrum antimicrobial latex paint surfaces employing active amphiphilic compounds. *ACS Appl. Mater. Interfaces* **2011**, *3*, 2878–2884. [[CrossRef](#)]
18. Makarewicz, E.N.; Shiichuk, A.V.; Syrotyn'ska, I.D. Quaternary ammonium salts as antimicrobial additives to water-dispersible paints. *Russ. J. Appl. Chem.* **2011**, *84*, 888–891. [[CrossRef](#)]
19. Goodwin, J.W.; Ottewill, R.H.; Pelton, R. Studies on the preparation and characterization of monodisperse polystyrene latices V.: The preparation of cationic latices. *Colloid Polym. Sci.* **1979**, *257*, 61–69. [[CrossRef](#)]
20. Antonietti, M.; Lohmann, S.; Van Niel, C. Polymerization in microemulsion. 2. Surface control and functionalization of microparticles. *Macromolecules* **1992**, *25*, 1139–1143. [[CrossRef](#)]
21. Kong, X.Z.; Zhu, X.; Jiang, X.; Li, X. Preparation and full characterization of cationic latex of styrene–butyl acrylate. *Polymer* **2009**, *50*, 4220–4227. [[CrossRef](#)]
22. Liu, Z.; Xiao, H.; Wiseman, N. Emulsifier-free emulsion copolymerization of styrene with quaternary ammonium cationic monomers. *J. Appl. Polym. Sci.* **2000**, *76*, 1129–1140. [[CrossRef](#)]
23. Naves, A.F.; Palombo, R.R.; Carrasco, L.D.M.; Carmona-Ribeiro, A.M. Antimicrobial Particles from Emulsion Polymerization of Methyl Methacrylate in the Presence of Quaternary Ammonium Surfactants. *Langmuir* **2013**, *29*, 9677–9684. [[CrossRef](#)] [[PubMed](#)]
24. Sanches, L.M.; Petri, D.F.S.; de Melo Carrasco, L.D.; Carmona-Ribeiro, A.M. The antimicrobial activity of free and immobilized poly (diallyldimethylammonium) chloride in nanoparticles of poly (methylmethacrylate). *J. Nanobiotechnol.* **2015**, *13*, 58. [[CrossRef](#)] [[PubMed](#)]
25. Melo, L.D.; Palombo, R.R.; Petri, D.F.S.; Bruns, M.; Pereira, E.M.A.; Carmona-Ribeiro, A.M. Structure–Activity Relationship for Quaternary Ammonium Compounds Hybridized with Poly(methyl methacrylate). *ACS Appl. Mater. Interfaces* **2011**, *3*, 1933–1939. [[CrossRef](#)] [[PubMed](#)]
26. Pereira, E.M.A.; Kosaka, P.M.; Rosa, H.; Vieira, D.B.; Kawano, Y.; Petri, D.F.S.; Carmona-Ribeiro, A.M. Hybrid Materials from Intermolecular Associations between Cationic Lipid and Polymers. *J. Phys. Chem. B* **2008**, *112*, 9301–9310. [[CrossRef](#)]
27. Ramos, J.; Forcada, J.; Hidalgo-Alvarez, R. Cationic Polymer Nanoparticles and Nanogels: From Synthesis to Biotechnological Applications. *Chem. Rev.* **2014**, *114*, 367–428. [[CrossRef](#)]
28. Wojciechowski, K.; Kaczorowski, M.; Mierzejewska, J.; Parzuchowski, P. Antimicrobial dispersions and films from positively charged styrene and acrylic copolymers. *Colloids Surf. B Biointerfaces* **2018**, *172*, 532–540. [[CrossRef](#)]
29. Van der Kooij, H.M.; Sprakel, J. Watching paint dry; more exciting than it seems. *Soft Matter* **2015**, *11*, 6353–6359. [[CrossRef](#)]
30. Steward, P.A.; Hearn, J.; Wilkinson, M.C. An overview of polymer latex film formation and properties. *Adv. Colloid Interface Sci.* **2000**, 195–267. [[CrossRef](#)]
31. Kiil, S. Drying of latex films and coatings: Reconsidering the fundamental mechanisms. *Prog. Org. Coat.* **2006**, *57*, 236–250. [[CrossRef](#)]
32. Keddie, J.L. Film formation of latex. *Mater. Sci. Eng. R Rep.* **1997**, *21*, 101–170. [[CrossRef](#)]
33. Lyklema, J. Surface charges and electrokinetic charges: Distinctions and juxtapositionings. *Colloids Surf. A Physicochem. Eng. Asp.* **2011**, *376*, 2–8. [[CrossRef](#)]
34. Bergström, L. Hamaker constants of inorganic materials. *Adv. Colloid Interface Sci.* **1997**, *70*, 125–169. [[CrossRef](#)]
35. Farrokhpay, S. A review of polymeric dispersant stabilisation of titania pigment. *Adv. Colloid Interface Sci.* **2009**, *151*, 24–32. [[CrossRef](#)] [[PubMed](#)]
36. Elzbiaciak-Wodka, M.; Popescu, M.N.; Ruiz-Cabello, F.J.M.; Trefalt, G.; Maroni, P.; Borkovec, M. Measurements of dispersion forces between colloidal latex particles with the atomic force microscope and comparison with Lifshitz theory. *J. Chem. Phys.* **2014**, *140*, 104906. [[CrossRef](#)] [[PubMed](#)]

