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Modeling Viruses' Isoelectric Points as a Milestone in Intensifying the Electrocoagulation Process for Their Elimination

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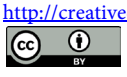

Abstract

In both nature and physicochemical treatment, virus end depends on electrostatic interplays. Suggesting an exact method of predicting virion isoelectric point (IEP) would assist to comprehend and predict virus end. To predict IEP, an easy method evaluates the pH at which the total of charges from ionizable amino acids in capsid proteins reaches zero. Founded on capsid charges, however, predicted IEPs usually diverge by some pH units from experimentally measured IEPs. Such disparity between experimental and predicted IEP was ascribed to the electrostatic neutralization of predictable polynucleotide-binding regions (PBRs) of the capsid interior. In the first part of this work, models assuming the 1) impact of the viral polynucleotide on the surface charge, or 2) contribution of only exterior residues to surface charge are discussed. Such models are relevant to non-enveloped viruses only, and an identical model for enveloped viruses remains difficult by the deficiency of information on enveloped virus IEP and uncertainties concerning the effect of the phospholipid envelope on charge and ion gradients. It is difficult now that modeling IEPs for viruses could be employed in assessing the needed electric field application during electrocoagulation (EC) process. Parameters such as pH and aqueous matrix greatly influence IEPs and EC.

Subject Areas

Chemical Engineering & Technology

Keywords

Virus, Isoelectric Point (IEP), Electrophoretic Mobility (EM), Electrocoagulation (EC), Electrochemical Disinfection (ED), Electric Field (EF)

1. Introduction

In virus behavior and movement in natural and engineered mediums, electrostatic forces have a crucial contribution [1]. In water, the electric charge of organic macromolecules is a function of the ionic force and pH [2]. For such a reason, it is commodious to evaluate the isoelectric point (IEP) of the virus [3]. At the IEP, the net electric charge of the virion net charge is neutral (zero), when determining the possible impacts of electrostatic forces. Above their IEP, organic particles (like virions) possess a net negative charge because of deprotonated carboxyl groups; however, below the IEP, protonated amine groups give a net positive charge. Without taking into account the charge quantity, determining just the sign of a viral particle's charge could tell water treatment (like coagulation [4] [5], disinfection [6] [7], or membrane filtration [8]), modeling virus transfer across porous media [9], and virus sampling and level [10] [11] [12] [13].

Electrostatic forces are not the only decisive factor of virus behavior and transfer [1]. Indeed, additional interactions (like van der Waals forces, the hydrophobic impact, cation bridging, and steric interactions) have as well a crucial contribution in virus interplays with the surrounding medium [4] [8] [14]. On the other hand, IEP is not an ideal measure of electrostatic forces under all circumstances. At a distance from the IEP, electrophoretic mobility (EM) is very dependent on environmental circumstances like conductivity [8]. The IEP could not specify if a particular virus can shift conclusively between strong positive and negative surface charges below and above the IEP, or turn near zero charges over a wide pH range. Whereas electrostatic forces are not an ideal predictor of virus physical/chemical interactions (and IEP is not an ideal measure of electrostatic forces in all circumstances), IEP gives a valid and quantitative benchmark for contrasting ecological interplays of unlike viruses over a set of circumstances and exploratory techniques. Further, focusing on IEP permits handling the biggest distinctions between theoretical and empirical findings before suggesting a more adopted model to evaluate the extents of surface charge and potential.

A large numbers of trials have been performed to model the IEP of non-enveloped viruses founded on ionizable residues within capsid proteins [15] [16] [17] [18] [19]. Nevertheless, important contradictions appear between predicted IEPs founded on capsid proteins and empirically evaluated virus IEPs. Whilst empirical IEPs are frequently noted in the acidic interval (pH 2 - 5) [20], capsid proteome sequences often include equiponderant levels of amino acids mirroring pre-

dicted IEPs near neutral pH [21]. Consequently, capsid amino acid composition alone could not explain virus IEP [1].

Numerous scientists have suggested electrostatic models of the virion to interpret the poor predictive level of ionizable amino acids [1]. Founded on a "soft colloid" model suggested by Duval and Ohshima [22], researchers [1] proposed that nucleic acids at the core of the virus capsid participate to overall virus surface charge. Researchers [19] presented an identical permeable virion model that measured the impact of capsid moieties founded on electrostatic screening of the surrounding environment. Both models propose that with augmenting permeability, buried components of the virion possess a bigger effect on the overall IEP [1] [19]. On the other hand, scientists [16] [18] proposed that only exterior residues participate to the surface charge; therefore, heterogeneous distribution of positive and negative amino acid charges inside the capsid coat lead to greater or lower IEP levels. Božič *et al.* [17] also estimated a one- or two-shell model of virion surface charge to explain heterogeneity in ionizable amino acid distribution, still the model was particularly used to experimenal IEP estimates only for bacteriophage PP7 [23]. Whereas the discussion concerning basic assumptions could be polarizing, not all parts of such models are opposed.

Lately, Heffron and Mayer [2] [24] proposed a differing procedure to modeling non-enveloped virion IEP founded not on a sole electrostatic model of the virion, but rather on the changing magnitude of electrostatic interplays between the capsid and the viral genome. As many of the viruses with the biggest difference between predicted and empirical IEPs displayed great capsid regions dedicated to binding the viral polynucleotide, Heffron and Mayer [2] assumed that the charges of these polynucleotide-binding regions (PBRs) and bound sections the viral polynucleotide itself are mutually neutralized. Heffron and Mayer [2] also predicted the location of PBRs from virus capsid proteome sequences to predict the IEP of viruses whose detailed capsid structures were obscure. Such procedure advocated remarks of Šiber *et al.* [25] that the two-shell model of Božič *et al.* [17] was convenient for spontaneously assembling viruses with strong, non-specific interactions between capsid proteins and ssRNA. Nevertheless, the PBR exclusion procedure displayed amelioration in IEP prediction for dsDNA viruses as well as ssRNA viruses [1] [2].

Heffron and Mayer [1] assessed the capacity of polynucleotide effect and exterior residue theories for suggesting a model of non-enveloped, icosahedral virus IEPs, as juxtaposed to the freshly presented hypothesis of PBR exclusion. They reviewed models proposing polynucleotide impact in light of empirical evidence. They examined the theory that external capsid residues participate disproportionately to global charge employing 3D capsid structures for 26 viruses with known (empirical) IEPs. They examined Heffron and Mayer's procedure of excluding PBRs, as well as the model of virion charge structure that arises from the PBR exclusion procedure. They examined the possibility of such competing theories for suggesting a predictive IEP model, as well as the impediments to implementing such a model to enveloped viruses.

This work discusses supplementary considerations for a predictive IEP model especially those related to enveloped viruses and interactions between viruses and the surrounding medium. A special interest is accorded to electrocoagulation (EC) process intensification for better eliminating viruses. A brief description is given about similarities related to charge neutralization of natural organic matter (NOM) and viruses. As an illustration of the successful implementation of the EC process, a brief review of this technique as a tertiary treatment of municipal wastewater is presented focusing on microbial removal pathways. A question is suggested and replied about if viruses' IEPs modeling will be employed in assessing the needed electric field (EF) application.

2. Additional Considerations for a Predictive Isoelectric Point (IEP) Model

2.1. Considerations for Enveloped Viruses

Actual models of virion charge stay restricted to non-enveloped, icosahedral virions [1]. Ecological persistence of viruses with phospholipid envelopes, like severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [26] [27] [28], is usually regarded as not enough to be important to transport or water and wastewater treatment [29] [30] [31]. For such cause, only non-enveloped viruses were taken into account here, excepting bacteriophages PM2 [PM2] and PRD1 [PRD1], which carry an internal lipid membrane [32] [33]. Nevertheless, several enveloped viruses, particularly those transmitted through the fecal-oral way (like avian influenza virus), could persevere for months in aqueous habitats [34] [35] [36]. Moreover, the electrostatic charge of enveloped viruses could tell virus removal through air filtration and deposition on surfaces [37] [38].

Regrettably, enveloped viruses constitute unique dares to IEP prediction [1]. Envelope phospholipids could participate considerably to surface charge, and the low dielectric constant of phospholipid bilayers could lower their apparent pK_a by as much as one pH unit [39]. The variety of phospholipids in virus envelopes can as well challenge efforts for a predictive model. Researchers [40] quantified over 125 various phospholipids from three strains of influenza virus and discovered that the composition of lipids in the virion envelopes varied not only from the host cell membrane but as well between virus strains. Because these phospholipids are obtained from the host, the complex lipid profiles are not predictable from the viral genome. Virions could earn else materials from the host as well. As an illustration, human papillomavirus obtains histones from the host that stabilize the polynucleotide inside the capsid [41]. Such structures are also not coded for in the viral genome, yet could affect global capsid charge via neutralizing the polynucleotide charge.

Probably most conclusive is that empirical IEP data for enveloped viruses are very sparse, with a poor agreement between sources [1]. Unluckily, only three genera are represented in Michen and Graule's exhaustive review of empirical IEP data [20]; however, IEPs of isolated proteins (and particularly glycoproteins)

from enveloped viruses are more frequent [1]. Empirical IEPs for numerous strains of *Orthopoxvirus* are obtainable; however, much of the data comes from two research groups with a poor agreement, even when juxtaposing the same virus strains [42]. As before noted for non-enveloped viruses [2], IEP quantifications founded on EM were more acidic than quantifications performed by isoelectric focusing or else manners. Nevertheless, the manner of quantification was confused by the source. Douglas *et al.* [43] [44] carried out the majority of enveloped virus EM quantifications, while Mouillot and Netter [45] were in charge of the majority of isoelectric focusing quantifications [20] [42]. As mentioned by Michen and Graule [20], Douglas *et al.* [43] [44] employed a more accurate purification technique than Mouillot and Netter [45]. Nevertheless, Douglas *et al.* [43] [44] carried out tests in molar sucrose, which may have influenced virion charge, aggregation, and EM [44]. Consequently, it is hard to decide if there is a veritable difference between the two procedures. Moreover, poxviruses could possess diverse infectious forms and several membrane-embedded proteins [46]. For suggesting a theory of enveloped virus IEP, the prime concern has to be gathering empirical IEP estimates for strains of viruses with one or two well-defined membrane proteins (like coronaviruses or influenza A virus [47] [48]). Nevertheless, the broad variety in envelope proteins between strains could still constitute a dare to extrapolation of a model to novel viruses.

2.2. Interactions between Viruses and the Surrounding Medium

In the water matrix, ions could bind to moieties on the capsid surface, that way changing surface charge [1]. This is particularly correct for multivalent ions like calcium and phosphate [20] [49], which could even be kept after viruses are transferred from the propagation/storage solution [9]. In addition to ions from the surrounding environment, polyvalent cations are inherent to the structure of several viruses. Such ions could greatly modify IEP, and can be so inherent as to be eliminated only by way of denaturation [50]. Among the viruses, five viruses (BP29, CPaV2, PM2, REO3, and SRVA) possess zinc, magnesium, and/or calcium-binding sites listed in the UniProt database (**Table 1**) [51]. Particularly, Simian rotavirus A (SRVA) possessed numerous cation-binding sites that can participate to the higher than predicted IEP. Such inherent ions, in addition to polyvalent counterions kept in the core, may possess a substantial influence on the global charge of several viruses. Nevertheless, the level to which such cations modify surface charge, as well as the irreversibility of numerous cation-binding sites, stays to be evaluated.

Virions could as well possess a more nuanced permeability than models of soft or hard colloids [1]. As an illustration, many viruses (like human rhinovirus, southern bean mosaic virus and *Mengo encephalomyocarditis* virus) possess selective cation channels located at capsid vertices [54] [55]. Further, bacteriophage MS2 possesses pores at its fivefold axes that are ringed by disordered loops with a single glutamic acid at the apex [1]. The negative charge of these loops above pH 4 could assist in selective diffusion of cations into the virion

Table 1. Classification and abbreviations for viruses, as previously employed in Heffron and Mayer [2].

Abbreviation	Species	Genus	Nucleic Acid	NCBI Taxon[1]	PDBID[2]	Resolution (Å)
AAV4	Adeno-associated virus 4	Dependoparvovirus	ssDNA	57,579	2g8g	3.2
BDMV	Belladonna mottle virus	Tymovirus	ssRNA	12,149	-	-
BP29	*Bacillus* phage Φ29	Salasvirus	dsDNA	10,756	-	-
CCMV	Cowpea chlorotic mottle virus	Bromovirus	ssRNA	12,303	1cwp	3.2
CMV	Cucumber mosaic virus	Cucumovirus	ssRNA	12,307	1f15	3.2
CPaV2	Canine parvovirus 2	Protoparvovirus	ssDNA	10,790	-	-
CPaV2[3]	Feline panleukopenia virus	Protoparvovirus	ssDNA	10,787	1c8g	3.0
CRPV	Cottontail rabbit papillomavirus	Kappapapillomavirus	dsDNA	31,553	-	-
CRPV[3]	Human papillomavirus 16	Alphapapillomavirus	dsDNA	333,760	5keq	4.3
CXA21	Coxsackievirus A21	Enterovirus	ssRNA	12,070	1z7s	3.2
CXB5	Human coxsackievirus B5	Enterovirus	ssRNA	103,907	-	-
CXB5[3]	Human coxsackievirus B3	Enterovirus	ssRNA	103,904	1cov	3.5
EBFR	Enterobacteria phage fr	Levivirus	ssRNA	12,017	1frs	3.5
EBGA	Enterobacteria phage GA	Levivirus	ssRNA	12,018	1gav	3.4
EBMS2	Enterobacteria phage MS2	Levivirus	ssRNA	329,852	2ms2	2.8
EBQB	Enterobacteria phage Qβ	Allolevivirus	ssRNA	39,803	5vly	3.3
EBSP	Enterobacteria phage SP	Allolevivirus	ssRNA	12,027	-	-
ECV1	Echovirus 1	Enterovirus	ssRNA	103,908	1ev1	3.6
ELV	Erysimum latent virus	Tymovirus	ssRNA	12,152	-	-
HAdV5	Human adenovirus 5	Mastadenovirus	dsDNA	28,285	4v4u	10
HHAV	Hepatitis A virus	Hepatovirus	ssRNA	12,098	4qpi	3.0
HRV2	Human rhinovirus 2	Enterovirus	ssRNA	12,130	1fpn	2.6
MEV	Mengo encephalomyocarditis virus	Cardiovirus	ssRNA	12,107	2mev	3.0

Continued

NOR1	Norwalk Virus	Norovirus	ssRNA	524,364	1ihm	3.4
PHIX	Enterobacteria phage ΦX174	Sinsheimervirus	ssDNA	10,847	2bpa	3.0
PM2	Pseudoalteromonas phage PM2	Corticovirus	dsDNA	10,661	2w0c	7.0
POL1	Poliovirus	Enterovirus	ssRNA	12,081	1hxs	2.2
PRD1	Enterobacteria phage PRD1	Alphatectivirus	dsDNA	10,658	1w8x	4.2
RCNM	Red clover necrotic mosaic virus	Dianthovirus	ssRNA	12,267	6mrm	2.9
REO3	Reovirus 3	Orthoreovirus	dsRNA	10,886	2cse	7.0
SBMV	Southern bean mosaic virus	Sobemovirus	ssRNA	652,938	4sbv	2.8
ScrMV	Scrophularia mottle virus	Tymovirus	ssRNA	312,273	-	-
SRVA	Simian rotavirus A	Rotavirus	dsRNA	450,149	4v7q	3.8
TBMV	Tobacco mosaic virus	Tobamovirus	ssRNA	12,243	-	-
TYMV	Turnip yellow mosaic virus	Tymovirus	ssRNA	12,154	1auy	3.0

[1]NCBI Taxon: National Center for Biotechnology Information (www.ncbi.nlm.nih.gov) taxonomical ID [52]. [2]PDBID: Protein Data Bank (rcsb.org) ID used for 3D structural comparisons [53]. [3]Alternate species/strain used for 3D structure only.

core, and can aid in recruiting and holding counterions to stabilize the negatively charged polynucleotide. This pathway would further demonstrate the shortage of impact of the viral genome on virion charge.

All the preceding factors may complicate a predictive model of virus IEP [1]. Whether a model could successfully combine or safely ignore such virion complexities constitutes significant future research. Nevertheless, every confounding factor for a single model of virion charge lends support for a procedure such as PBR exclusion that identifies functional virion structures rather than universally applying a simplified physical model. With expanded empirical IEP data, more accurate IEP prediction can be feasible founded on conserved virion structures. The PBR exclusion model possesses implementations in water and wastewater treatment, as well as virus transport and microbial source tracking. As a general heuristic, viruses relying on electrostatic interactions between the polynucleotide and capsid proteins are more probable to possess acidic IEPs outside the circumneutral range expected from the sum of ionizable capsid residues. Therefore, scientists could profit from the insights of the PBR exclusion method, even without identifying known PBRs or employing the PBR prediction method suggested by Heffron and Mayer [2]. Future research has to merge PBR exclusion into a quantitative model for virus surface charge. Moreover, the PBR exclusion method furnishes rise to a conceptual electrostatic model of the virion that better

unifies empirical evidence of virion structure and morphogenesis. Such a conceptual model is not an *ab ovo* assumption to account for a small subset of aberrant viruses. Instead, the PBR model follows from the success of the PBR exclusion method in accounting for both empirical IEPs that align with capsid residue composition, as well as empirical IEPs that change considerably. Additional confirmation and refinement of this electrostatic model, particularly concerning the ionic composition of the virion core, could have far-reaching importance for structural virology in general [1].

3. Electrocoagulation (EC) Process Intensification for Better Killing Viruses

In the field of killing pathogens existing in water, if there is a method that has attracted huge attention from water treatment specialists it is the electrocoagulation (EC) process [56]. During the last two decades and thanks to its techno-economic benefits, this electrochemical technology has been the subject of many hundreds of researches and patents published throughout the entire world [57] [58]. The generally accepted tendency concerning the usage of the EC technique is to employ it as an integrated step with additional processes [59]. In the field of killing microorganisms, EC process is frequently inserted as a pre-stage before electrooxidation (EO) method in the treatment train [60] [61]. For such a combination, more important virus reduction is possibly reached via the collective actions of physical removal by coagulation/filtration, ferrous iron-based disinfection [62] [63] [64], and EO disinfection [65]. In this context, much more research needs to be realized to distinguish among the electric field (EF) and cohesion contributions [66]. Furthermore, more investigation has to be pointed on evaluating the more and more probable production of the hydroxyl radical ($^{\bullet}$OH) during the EC technology [67] [68] [69]. Like in the chemical water disinfection, on the other hand, identical problems such as disinfection by-products (DBPs) generation have also appeared in the EC applications [70] [71] [72]. More research needs to be pointed into such directions [73] [74] [75].

As mentioned above, bacteria and viruses show a tendency to adsorb onto surfaces such as activated carbon, fibrous carbon, or ion exchange resins. This tendency is driven mainly by electrostatic forces between charged groups on the cell wall and on the adsorbent [57].

Several mechanisms have been proposed to account for the lethality of electrochemical exposure including: 1) oxidative stress and cell death due to electrochemically generated oxidants ("killer" agents such as $^{\bullet}$OH), 2) irreversible permeabilisation of cell membranes by the applied EF [76] [77] [78], and 3) electrochemical oxidation of vital cellular constituents during exposure to electric current [79] or induced EFs [57] [80] [81].

Throughout the EC method, employing Fe/Al anodes, physical elimination and chemical deactivation pathways are suggested for bacteria reduction procedure: 1) entrapping pathogens in flocs, 2) destabilizing negatively charged microbes through *sweep* flocculation [82], and 3) demobilizing bacteria cell enve-

lopes upon electrochemically formed reactive oxygen species (ROSs) or direct impact of the EF [76] [83] [84] [85]. Deepest investigation works on microbes' removal through EC are more called to promote the industrial applications of this performant technology.

4. Similarities with Charge Neutralization of Natural Organic Matter (NOM) and Viruses

Since they are negatively charged, there are analogies between natural organic matter (NOM) and microorganisms in terms of their removal via charge neutralization mechanism during chemical coagulation (CC) and EC processes (**Figure 1** and **Figure 2**). EC as efficient technique in mineral and organic matters removal [86] [87] [88] has been proven also performant in pathogens (including *Escherichia coli* and viruses) removal [89]. Therefore, this electrochemical process is promising water treatment technology even if more studies must be done about the best choice of EC electrodes [90] [91]. For instance, anode in iron (Fe) and cathode in metal that does not produce chlorine in water stream to avoid DBPs formation since it was eventually demonstrated that even at very low chloride concentrations (less than 100 mg/L) sufficient free chlorine can be produced to efficiently disinfect water [57] [92] [93].

Several researchers focused on promoting the large industrial usage of EC as a green technology [94] [95] [96]. Concerning EC process design, the focus should be accorded to intensify the EC device in terms of residence time and close contact opportunities between water pollutants and electrodes area. The laminar *vs.* turbulent regime should be given more interests to better increase the metallic cations liberation from the anode and avoid or reduce the passivation of the electrodes. Evolution of hydrogen form cathode and oxygen from anode should be well optimized; at the same time, chlorine emanation from anode should be avoided or decreased to avoid DBPs generation [97] [98] [99]. Moreover, increasing

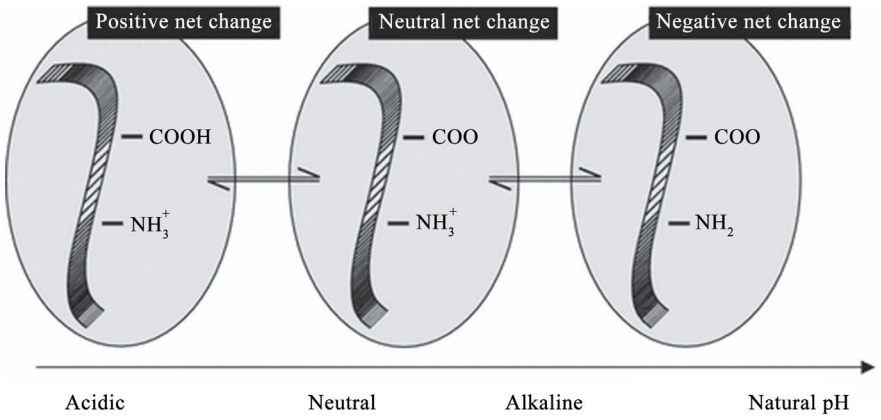

Figure 1. Schematic showing the protonation states of functional groups on a protein sector as a function of pH. The carboxyl and amino functional groups are in equilibrium with the H_3O^+ concentration and thus alter their charge if the environmental pH is changed. The net charge of a protein (or protein sector) is therefore determined by the superposition of the protonated and unprotonated states of its functional groups [20].

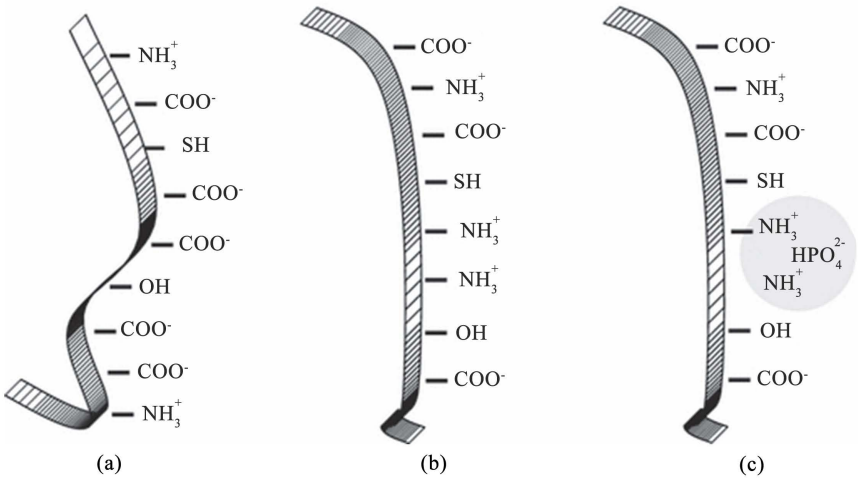

Figure 2. Sketches coat protein segments of different viruses and the arrangement of their functional groups. The environmental pH for all three schematic draws is assumed to be neutral. In (a) and (b), we compare the occurrence of different types of functional groups on two different coat proteins leading to a difference in virus isoelectric point (IEP). While in (a) the deprotonated carboxyl groups are superior, in (b) these negatively charged groups are balanced out by a relative higher number of protonated amino groups. Hence, (a) shows a section of a coat protein which belongs to a virion having an IEP in the acid regime, whereas the draw in (b) refers to a virion possessing an IEP at neutral pH. In (c) the identical coat protein is sketched as in (b) including the illustration of surface complexation or specific adsorption (marked with the gray circle) and thus the water chemistry-dependent IEP alteration. The effect is shown using a hydrogen phosphate ion that binds with their oxygen atoms to the hydrogen atoms of the amino functional group. Hence, neutralizing the prior positive charge and leading to a relative decrease in the IEP of the virion when compared with (b) [20].

the water temperature using solar energy heating would enhance the EC process efficiency technically, energetically, and economically [100]. The heated EC process combines EC with distillation (or its similar version, even if at low temperature between 20˚C - 100˚C) or membrane distillation using solar radiation [101] [102]. Finally, the EC method remains promising vis-a-vis pathogen removal and water treatment in a general manner [103] [104] [105].

5. Iron Electrocoagulation (Fe-EC) as a Tertiary Treatment of Municipal Wastewater

Bicudo *et al.* [106] estimated the possibility of low voltage iron electrocoagulation (Fe-EC) as a technology for treating municipal secondary effluent treatment. They concentrated on eliminating microbial indicators, antibiotic resistant bacteria (ARB), and nutrients. They employed charge dosage (CD) and charge dosage rate (CDR) as the major process control variables. Tests with synthetic secondary effluent illustrated > $4\log_{10}$ and >$5\log_{10}$ removal for phage ΦX174 and for *E. coli* WR1, respectively. In real effluents, bacterial indicator removal exceeded $3.5\log_{10}$, ARB were removed below detection limit (≥$2.5\log_{10}$), virus removal reached $2.3\log_{10}$ and *Clostridium perfringens* spore removal exceeded $2.5\log_{10}$. In both real and synthetic wastewater, trials depicted that bacterial removal augmented

with increasing CD and decreasing CDR. Virus elimination augmented with increasing CD even if it was irresponsive to CDR. Further, *C. perfringens* spore reduction increased with augmenting CD yet attained a removal plateau, being also irresponsive to CDR. Phosphate removal exceeded 99%, while total nitrogen and chemical oxygen demand removals were below 15% and 58%, respectively. Operational cost estimates were made for power and iron plate consumption, and were found to be in the range of 0.01 to 0.24 €/m^3 for the different assayed configurations. Consequently, low voltage Fe-EC is a promising technology for pathogen removal of secondary municipal effluents, with log 10 removals comparable to those obtained by traditional disinfection methods like chlorination, UV, or ozonation.

5.1. Influence of Water Matrix on Electrocoagulation (EC) Performance

Bicudo *et al.* [106] proved the impact of water matrix (synthetic *vs.* real secondary effluent) for bacteria and virus indicator elimination by EC, as well as nutrient reduction. In real secondary effluents, *E. coli* reduction was $1 - 2\log_{10}$ smaller than that noted for *E. coli* in synthetic effluents, even when the Fe injection was doubled. Identical remarks were noted concerning phage ΦX174, with reduction also dropping by $1 - 2\log_{10}$ in real secondary effluents. Even if the response reached with synthetic and real secondary effluent was identical in qualitatively, reduction attained for *E. coli* and phage ΦX174 still differs by orders of magnitude.

It was suggested that the complexity of the water matrix from real secondary effluents, and its bigger level of organic matter, iron-scavenging anions and complexation agents (like phosphates, citrates, carbonates and sulfates) are in charge of considerably reduced coagulant formation or microbial eliminations [5] [84] [106] [107].

Such chemicals are known to be in charge of hindering Fe^{2+} oxidation into dissolved Fe^{3+}, thus reducing coagulant precipitation and subsequent sweep flocculation [106]. Anfruns-Estrada *et al.* [108] only estimated *C. perfringens* spore reduction in real secondary effluents, illustrating identical features quantitatively and qualitatively with previous Fe-EC research performed in real sewage and secondary effluents.

5.2. Microbial Removal Pathways

Bicudo *et al.* [106] noticed that eliminating all bacterial indicators was globally identical without taking into account their resistance to antibiotics or their Gram classification, with the reduction being highly dependent on the quantity and speed of Fe liberation. Identical remarks remain as well true for somatic coliphages (even if seeming less sensitive to the rate of dosage), even if do not totally apply for *C. perfringens* spores. This indicates a variable response to the Fe-EC process for each type of microorganism.

Bicudo *et al.* [106] recognized three routes for pathogens removal, that is 1)

enmeshment (or adsorption) in the $Fe(OH)_{2(s)}/Fe(OH)_{3(s)}$, and elimination via deposition; 2) demobilization due to generation of ROSs or killing agents; and 3) killing due to EF. Enmeshment is largely viewed as the controlling reduction route of microorganisms [83] [84] [106] [109] [110]. This is widely attributed to the affinity of their surface functional groups, like teichoic acids and phospholipids, with the EC flocs. Such functional groups are observed in identical quantities in Gram-positive and Gram-negative bacterial cell walls [109]. Virus elimination has been assigned to both $Fe(OH)_{2(s)}/Fe(OH)_{3(s)}$ enmeshment [5] [111], and demobilization via either ROSs or chlorine-based oxidants produced through anodic reduction [5] [106] [112].

As mentioned by Bicudo *et al.* [106] concerning CDR contribution in favoring either of the previously mentioned removal route, Heffron *et al.* [113] matched Fe^{2+} oxidation rate and bacteriophage elimination. Quick oxidation of Fe^{2+} conducts to a shorter exposure period and thus poorer contact between the phages and the reactive iron species, resulting in a less significant killing [113]. Such findings suggest that ROSs formed during Fe^{2+} oxidation are a prime contributor in killing pathogens throughout Fe-EC, with the influence of these being stronger for slowly occurring oxidations. Bicudo *et al.* [106] noticed that the adopted overnight settling for all tests probably affects $O_{2(g)}$ diffusion into the effluent enhancing the slow Fe^{2+} oxidation, thus considerably influencing the elimination. Bicudo *et al.* [106] noted for both synthetic and real effluents an augmenting reduction performance for bacterial and viral indicators under reducing CDRs (less important for viruses), even when the quantity of liberated iron was similar. The noticed dependency of microorganisms' elimination on CDR proposes that the formation of ROSs may be really a controlling parameter in the course of Fe-EC. During the anode oxidation, the aerobic oxidation of Fe^{2+} liberated generates a series of reactive species that comprises superoxide ion ($^{\bullet}O_2^-$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($^{\bullet}OH^-$) [114] [115], all of which are renowned to possess killing potentials [111] [112]. This involves that microbial elimination by Fe-EC could be really an integration of physical separation and chemical demobilization, and not just an adsorption-sedimentation process [106]. It can as well clarify why spores (dormant bacterial structures, highly resistant to chemical attack) are notably less touched than bacterial indicators by changing CD or CDR [106].

Researchers [69] discussed the advanced oxidation process (AOP) phenomena in EC process. AOPs have been widely described as near ambient temperature treatment techniques founded on highly reactive radicals, especially $^{\bullet}OH^-$ as the main oxidant. Since water-containing colloidal particulates, oils, or other contaminants move through the applied EF, there may be ionization, electrolysis, hydrolysis, and free-radical formation that could modify the physicochemical characteristics of water and pollutants. When the EC device run at a high cell potential and an anodic process takes place in the potential region of water discharge, $^{\bullet}OH^-$ is produced. To increase considerably the possibilities to form free radicals during EC, ultrasound coupled with EC could be very helpful as noted

by some scientist [69]. Moreover, EC process at pH ≤ 3 possesses more probability to generate hydroxyl radicals. Table 2 presents the detailed Fe-EC reactions in the case of Fe (and Al for comparison purpose) [61]. At pH at pH ≤ 3 (Table 2), $O_{2(g)}$ is generated besides Fe^{2+} liberation at the anode. Such conditions seem to be more favorable to $^{\bullet}OH^{-}$ production even if there is no evidence of the occurrence of AOP phenomena and more research remains required dealing with free radicals generation in EC process [60] [67] [68] [69].

6. Using Viruses' Isoelectric Points (IEPs) Modeling in Assessing the Needed Electric Field (EF) Application

As seen above, the EF contribution in ED generally and EC particularly remains fundamental since EF is poisonous to microbial cells [76] [116]. Microorganisms are electrically charged, like NOM, this why the EF action is fundamental in EC process [117] [118]. As shown previously, modeling IEPs for viruses is crucial in understanding their behavior in aquatic medium. Evaluating the required EF application, in terms of intensity and residence time, for their elimination during electrochemical treatment is also important. A question may arise here: what if modeling IEPs for viruses will be employed in assessing the needed EF application?

In this direction, Heffron *et al.* [5] estimated human virus alleviation and quantitatively evaluated the death of viruses in Fe-EC (Figure 3). They affirmed that the complexity of natural water matrices deserves more experimentation of

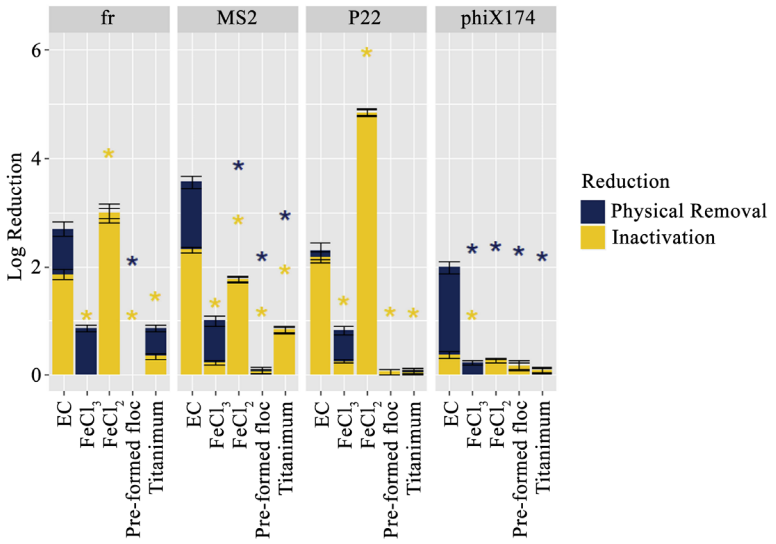

Figure 3. Mechanisms of bacteriophage mitigation due to electrocoagulation (EC), chemical coagulation (CC), adsorption and electrooxidation (EO). Inactivation and physical removal were compared between EC, CC with ferric chloride ($FeCl_3$), CC with ferrous chloride ($FeCl_2$), flocs formed by EC prior to the addition of bacteriophages (pre-formed floc), and EO with inert titanium electrodes (Titanium). Asterisks indicate a significant difference in log reduction from EC due to physical removal (blue asterisk) or inactivation (yellow asterisk). Error bars represent standard error of the mean of triplicate tests [5].

Table 2. EC mechanisms using Fe (pH 2, 7 and 12) and Al (pH 7) electrodes [61].

Fe mechanisms	
Mechanism #1 (pH 2)	Anode: $2Fe_{(s)} - 4e^- \rightarrow 2Fe^{2+}_{(aq)} \quad (E° = +0.447\ V)$ (1) $2H_2O_{(l)} - 4e^- \rightarrow O_{2(g)} + 4H^+_{(aq)} \quad (E° = -1.229\ V)$ (2) Solution: $2Fe^{2+}_{(aq)} + 4OH^-_{(aq)} \rightarrow 2Fe(OH)_{2(s)}$ (3) Cathode: $8H^+_{(aq)} + 8e^- \rightarrow 4H_{2(g)} \quad (E° = 0.000\ V)$ (4) Total: $2Fe_{(s)} + 6H_2O_{(l)} \rightarrow O_{2(g)} + 4H_{2(g)} + 2Fe(OH)_{2(s)}$ (5)
Mechanism #2 (pH 7)	Anode: $2Fe_{(s)} - 4e^- \rightarrow 2Fe^{2+}_{(aq)} \quad (E° = +0.447\ V)$ (1) $Fe^{2+}_{(aq)} - e^- \rightarrow Fe^{3+}_{(aq)} \quad (E° = -0.771\ V)$ (6) $Fe_{(s)} - 3e^- \rightarrow Fe^{3+}_{(aq)} \quad (E° = +0.037\ V)$ (7) Solution: $Fe^{2+}_{(aq)} + 2OH^-_{(aq)} \rightarrow Fe(OH)_{2(s)}$ (3) $2Fe^{3+}_{(aq)} + 6OH^-_{(aq)} \rightarrow 2Fe(OH)_{3(s)}$ (8) Cathode: $8H_2O_{(l)} + 8e^- \rightarrow 4H_{2(g)} + 8OH^-_{(aq)} \quad (E° = -0.828\ V)$ (9) Total: $3Fe_{(s)} + 8H_2O_{(l)} \rightarrow Fe(OH)_{2(s)} + 2Fe(OH)_{3(s)} + 4H_{2(g)}$ (10)
Mechanism #3 (*pH 12*)	Anode: $2Fe_{(s)} - 6e^- \rightarrow 2Fe^{3+}_{(aq)} \quad (E° = +0.037\ V)$ (7) Solution: $2Fe^{3+}_{(aq)} + 6OH^-_{(aq)} \rightarrow 2Fe(OH)_{3(s)}$ (8) Cathode: $6H_2O_{(l)} + 6e^- \rightarrow 3H_{2(g)} + 6OH^-_{(aq)} \quad (E° = -0.828\ V)$ (9) Total: $2Fe_{(s)} + 6H_2O_{(l)} \rightarrow 2Fe(OH)_{3(s)} + 3H_{2(g)}$ (11)
Al mechanism	
Mechanism (*pH 7*)	Anode: $Al_{(s)} - 3e^- \rightarrow Al^{3+}_{(aq)} \quad (E° = +1.66\ V)$ (12) $2H_2O_{(l)} - 4e^- \rightarrow O_{2(g)} + 4H^+_{(aq)} \quad (E° = -1.229\ V)$ (2) Solution: $Al^{3+}_{(aq)} + 3OH^-_{(aq)} \rightarrow Al(OH)_{3(s)}$ (12) $Al(OH)^-_{4(aq)} \rightarrow OH^-_{(aq)} + Al(OH)_{3(s)}$ (13) Cathode: $4H_2O_{(l)} + 4e^- \rightarrow 2H_{2(g)} + 4OH^-_{(aq)} \quad (E° = -0.828\ V)$ (9) $Al_{(s)} + 4OH^-_{(aq)} - 3e^- \rightarrow Al(OH)^-_{4(aq)}$ (14) Total: $2Al_{(s)} + 8H_2O_{(l)} \rightarrow 5H_{2(g)} + 2Al(OH)_{3(s)} + O_{2(g)}$ (15)

virus reduction in natural waters. In pointing out virus removal, ΦX174 was the only bacteriophage surrogate resistant to ferrous demobilization, probably because of electrostatic repulsion between ΦX174 and Fe^{2+} at pH 6 and/or shielding of ΦX174 virions in aggregates near neutral pH. Even if electrostatic interactions between Fe^{2+} and virions possibly interpret at least some of the distinctions in killing performance between viruses, resistant viruses also had thicker capsids. Heffron *et al.* [5] concluded that the shortage of measured IEP information for human viruses blocks a comprehensive examination of such supposition, even if an exhaustive theoretical estimation of capsid structure could furnish further comprehension where experimental procedures are restrictive.

To disinfect water, ultrafiltration (UF) has been shown to be performant; however, the technique was influenced by the aqueous matrix and thus, limited reduction of bacteriophage PP7 was reached [119]. The occurrence of divalent cations decreased the performance as compared to monovalent cations and species with amphoteric behavior like bicarbonate. Gentile *et al.* [119] found that size of the bacteriophage did not change greatly with pH or ionic strength. Besides, at circumnatural pH (*i.e.*, from 5 to 8) viruses constitute small aggregates, turning off UF treatment. Small energy barriers were reached for NaCl and $NaHCO_3$ at 100 mM. For 1 and 10 mM background solutions, electrostatic repulsion was anticipated. The viral elimination augmented in this order: Mg^{2+}, Ca^{2+}, and Na^{+} with HCO_3^-. For PP7, modifications in pH ranged between 5 and 8 (far from the virus IEP) or ionic strength did not change the modeling forecasts concerning stability and attachment. Such findings called attention to the significance of electrostatic repulsion in improving virus elimination by membrane filtration.

Concerning the question addressed above, it is difficult now that modeling IEPs for viruses could be employed in assessing the needed EF application. Controlling parameters such as pH and aqueous matrix, which is usually complicated due to NOM occurrence and metal-scavenging anions and complexation agents (like phosphates, citrates, carbonates and sulfates), remains difficult. Such chemicals, as mentioned above, are responsible of decreased coagulant generation or pathogens reduction [5] [84] [106] [107].

7. Conclusions

In both nature and physicochemical treatment, virus end depends on electrostatic interactions. Suggesting an exact method of predicting virion isoelectric point (IEP) would assist to comprehend and predict virus end. To predict IEP, an easy method evaluates the pH at which the sum of charges from ionizable amino acids in capsid proteins reaches zero. Founded on capsid charges, however, predicted IEPs usually diverge by some pH units from experimentally measured IEPs. Such disparity between experimental and predicted IEP was in fact ascribed to the electrostatic neutralization of predictable polynucleotide-binding regions (PBRs) of the capsid interior. In this work, models assuming the 1) effect of the viral polynucleotide on the surface charge, or 2) contribution of only exterior residues

to surface charge are discussed. Such models are relevant to non-enveloped viruses only, and an identical model for enveloped viruses remains complex by the deficiency of information on enveloped virus IEP and uncertainties concerning the effect of the phospholipid envelope on charge and ion gradients [1].

Concerning the interrogation if viruses' IEPs modeling will be employed in assessing the needed EF application, it is difficult now that modeling IEPs for viruses could be employed in assessing the needed EF application. Controlling parameters such as pH and aqueous matrix, which is usually complicated due to NOM occurrence and metal-scavenging anions and complexation agents (like phosphates, citrates, carbonates and sulfates), remains difficult. Such chemicals are responsible of decreased coagulant generation or pathogens reduction.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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