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REVIEW ARTICLE

Isoelectric points of viruses

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Summary

Viruses as well as other (bio-)colloids possess a pH-dependent surface charge in polar media such as water. This electrostatic charge determines the mobility of the soft particle in an electric field and thus governs its colloidal behaviour which plays a major role in virus sorption processes. The pH value at which the net surface charge switches its sign is referred to as the isoelectric point (abbreviations: pI or IEP) and is a characteristic parameter of the virion in equilibrium with its environmental water chemistry. Here, we review the IEP measurements of viruses that replicate in hosts of kingdom plantae, bacteria and animalia. IEPs of viruses are found in pH range from 1·9 to 8·4; most frequently, they are measured in a band of $3{\cdot}5 < \text{IEP} < 7$. However, the data appear to be scattered widely within single virus species. This discrepancy is discussed and should be considered when IEP values are used to account for virus sorption processes.

Introduction

Protonation of interfacial compounds of organic or inorganic nature in water leads to the formation of pH-dependent electrically charged surfaces that govern environmental processes (Gerba 1984; Brown *et al.* 1999; Al-Abadleh and Grassian 2003). The classic theory of Derjaguin, Landau, Verwey, and Overbeek (DLVO) takes into account Van der Waals and electrostatic interactions between colloidal particles and may be used to describe their sorption behaviour (Derjaguin 1934; Derjaguin and Landau 1941; Verwey and Overbeek 1948). If the net charge of the colloid is equal to zero at a particular pH, this electrically neutral state is termed an isoelectric point (IEP) (Parks 1965). The same term is used for bio-colloids such as bacteria, viruses and proteins. Figure 1 sketches a protein sector and illustrates the origin of its net surface charge which is because of a superposition of protonated and unprotonated states of functional groups. In the case of nonenveloped viruses, the functional groups of the coat protein determine the net surface charge of the virion to a great extent.

Surface charge of viruses plays a major role in various sorption processes. Schijven and Hassanizadeh (2000) elaborately reviewed the adhesion and transport phenomena of viruses in the subsurface. Adsorption to various soils was studied by Gerba (1984). In the field of water treatment, the virus' surface charge is used in flocculation processes (Matsushita *et al.* 2006) or filters working on the electrostatic adsorption principle (Wegmann *et al.* 2008a,b) to obtain safe drinking water. Virus concentration from large volumes of drinking water by adsorption to and subsequent elution from charged microporous filters is used as a detection method of waterborne viruses (Sobsey and Jones 1979; Cashdollar and Dahling 2006) and has been recommended by the United States Environmental Protection Agency in the Information Collection Rule (USEPA ICR). Virus characterization as well as purification was achieved with chromatofocusing based on the virus' IEP (Brorson *et al.* 2008). The technique of nanowire arrays enables the electrical detection of a single virus based on surface properties (Patolsky *et al.* 2004). All these processes are governed by electrostatic interactions. Hence, the IEP is a crucial value which at first glance gives the researcher an idea about the virus' surface charge in a certain environment and thus about its sorption behaviour. However, the authors noticed a great discrepancy in the literature when searching for IEP

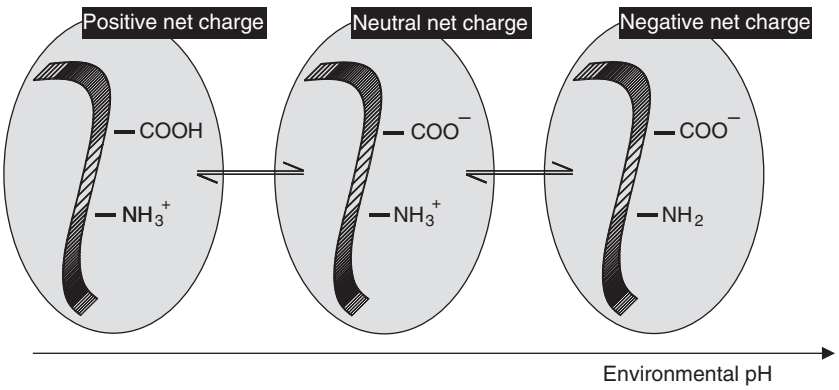

Figure 1 Schematic showing the protonation states of functional groups on a protein sector as a function of pH. The carboxyl and amino functional groups are in equilibrium with the H_3O^+ concentration and thus alter their charge if the environmental pH is changed. The net charge of a protein (or protein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

values of a single-virus species. Application of electrostatic theory to explain the adsorption behaviour of viruses on ceramic surfaces was thus unfeasible. Here, we review the published IEP values of viruses with the goal to reveal the source of discrepancy found in literature, analogous to the work of Kosmulski (2003) who found that IEP scattering of inorganic solid (hydr)oxides was mainly because of impurities. An earlier work has dealt with the IEP measurements of proteins (pI) by Righetti and Caravaggio (1976) who compiled values and discussed generally the potential sources of deviations.

Evaluation of literature

A total of 137 IEP measurements mainly found with the help of database libraries were available to the authors. These data refer to 104 viruses that differ in species and strain and were determined from 48 studies conducted since 1938. Virus classification was carried out according to the Universal Virus Database of the International Committee on Taxonomy of Viruses (ICTVdb) (ICTVdB – The Universal Virus Database 2002). Viruses were compiled in Table 1 and sorted alphabetically according to their host, species, and strain. This distinction between virus species and strain seems essential if one assumes that strains within a single species may possess modification in the coat proteins: As the coat protein partly defines the IEP of the virion, exchange of amino acids with other peptides owing different functional groups is expected to change the IEP of the whole virus particle. In Fig. 2a,b, sectors of two different coat proteins and their functional groups are sketched for illustration. Although not including recently demonstrated inner structural and chemical contribution to electrophoretic mobility (EM) of soft particles (Langlet *et al.* 2008a), Fig. 2a,b represents the base aspect of why viruses may own different IEPs.

After virus classification was completed, the IEPs of the viruses were added to Table 1 accompanied by their methods of determination. The majority of the measurement techniques used were based on either isoelectric focusing or EM. Chromatofocusing and electrical detection using nanowire field effect transistors (EDN-FET) as promising new techniques have also been applied. In some cases, simply the detection of virus aggregation as a function of pH leads to determination of virus neutral net charge. All measurement techniques are listed under methods, whereas question marks (?) indicate unknown measurement techniques.

An additional column was introduced into Table 1 that estimates the purity of the measured virus suspensions. This is a crucial point as it was found for inorganic solid materials in aquatic environment that the presence of impurities may alter the IEP (Kosmulski 2003). Crude, laboratory-made virus stock suspensions commonly contain cell debris of hosts as well as growth-stimulating agents such as nutrients. These additional substances are very likely to carry a surface charge and hence are able to disturb the measurement by two ways: (i) the additional substances appear in high concentration, and thus the reading corresponds rather to the additives than to the virus itself leading to an artefact; (ii) additional substances remain in lower concentration but interact with the virus' interface via specific adsorption (Douglas *et al.* 1966). Purity of virus suspension is thus of great importance and is scored within this study by the following terms: 'high' if several purification steps were undertaken, e.g. filtration – centrifugation – dialyses, or if the author(s) proofed isolation/purification experimentally. In case, the isolation of virus particles was performed rather inadequately, in terms of the above-mentioned definition, the column was filled with 'low'. Question marks indicate the publication of IEP measurements where purification was not addressed at all or inaccessible.

Table 1 Gives a literature overview on isoelectric points (IEP) of viruses. Viruses are listed and classified according to Universal Virus Database of the International Committee on Taxonomy of Viruses

Host kingdom	Virus species	Strain	IEP(s)	Method	Purity	Reference
Animalia	*Adeno-associated virus – 4*	Adeno-associated virus – 4	2·6	IEF-DA	High	Salo and Mayor (1978)
Animalia	*Alastrim*	Butler	3·4	EM-LM	High	Douglas *et al.* (1969)
Animalia	*Cowpox*	Brighton	4·3	EM-LM	High	Douglas *et al.* (1969)
Animalia	*Cowpox*	Brighton (egg)	4·3	EM-LM	High	Douglas *et al.* (1966)
Animalia	*Cowpox*	Brighton (rabit)	4·3	EM-LM	High	Douglas *et al.* (1966)
Animalia	*Cowpox*	Kampen	5·4	IEF-DA	Low	Mouillot and Netter (1977)
Animalia	*Cowpox*	Leuwarden	5·2	IEF-DA	Low	Mouillot and Netter (1977)
Animalia	*Encephalomyocarditis virus*	Mengovirus L	8·1 and 4·6	IEF-PA	High	Chlumecka *et al.* (1973)
Animalia	*Encephalomyocarditis virus*	Mengovirus M	4·4 and 6·3	IEF-PA	High	Chlumecka *et al.* (1973)
Animalia	*Encephalomyocarditis virus*	Mengovirus M	8·4 and 4·6	IEF-PA	High	Chlumecka *et al.* (1977)
Animalia	*Encephalomyocarditis virus*	Mengovirus S	4·6 and 6·8	IEF-PA	High	Chlumecka *et al.* (1973)
Animalia	*Feline panleukopenia virus*	Canine parvovirus	5·0	IEF-A	?	Weichert *et al.* (1998)
Animalia	*Hepatitis A virus*	Hepatitis A virus	2·8	IEF-DA	?	Nasser *et al.* (1992)
Animalia	*Human adenovirus C*	Human adenovirus 5	4·5	EM-LS	?	Trilisky and Lenhoff (2007)
Animalia	*Human enterovirus B*	Human coxsackievirus B 5	4·75 and 6·75	IEF-DA	Low	Butler *et al.* 1985
Animalia	*Human enterovirus B*	Human echovirus 1	5·6 and 5·1	IEF	?	Murray and Parks 1980
Animalia	*Human enterovirus B*	Human echovirus 1	4·0	IEF-DA	Low	Butler *et al.* (1985)
Animalia	*Human enterovirus B*	Human echovirus 1 (4CH-1)	5·5	IEF-A	?	Zerda and Gerba (1984)
Animalia	*Human enterovirus B*	Human echovirus 1 (R115)	6·2	IEF-A	?	Zerda and Gerba (1984)
Animalia	*Human enterovirus B*	Human echovirus 1 (V212)	6·4	IEF-A	?	Zerda and Gerba (1984)
Animalia	*Human enterovirus B*	Human echovirus 1 (V239)	5·3	IEF-A	?	Zerda and Gerba (1984)
Animalia	*Human enterovirus B*	Human echovirus 1 (V248)	5·0	IEF-A	?	Zerda and Gerba (1984)
Animalia	*Human enterovirus C*	Human coxsackievirus A 21	6·1 and 4·8	IEF	?	Murray and Parks (1980)
Animalia	*Human rhinovirus A*	Human rhinovirus 2	6·8	CIEF	Low	Schnabel *et al.* (1996)
Animalia	*Human rhinovirus A*	Human rhinovirus 2	6·4	IEF-DA	Low	Korant *et al.* (1975)
Animalia	*Influenza A virus*	H1N1 (Leningrad)	4·5, 4·35, 4·25, 4·0*	EM-LM	High	Molodkina *et al.* (1986)
Animalia	*Influenza A virus*	H3N1	6·5–6·8	IEF-PA	Low	Brydak (1993)
Animalia	*Influenza A virus*	H3N2 (Leningrad)	5·0	EM-LM	High	Molodkina *et al.* (1986)
Animalia	*Influenza A virus*	PR8	5·3	EM-LM	Low	Miller *et al.* (1944)
Animalia	*Influenza A virus*	Influenza A virus	6·5–7·0	EDN-FET	?	Patolsky *et al.* (2004)
Animalia	*Mammalian orthoreovirus*	Serotype 3 (Dearing)	3·8	EM-LM	Low	Taylor and Bosmann (1981b)
Animalia	*Mammalian orthoreovirus*	Serotype 3 (Dearing)	3·9	IEF-DA	Low	Floyd and Sharp (1978)
Animalia	*Monkeypox*	Chimpanzee Paris	6·2	IEF-DA	Low	Mouillot and Netter (1977)
Animalia	*Monkeypox*	Copenhague	6·5	IEF-DA	Low	Mouillot and Netter (1977)
Animalia	*Monkeypox*	Denmark	3·4	EM-LM	High	Douglas *et al.* (1969)
Animalia	*Neuro-Vaccinia*	Levaditi	4·2	EM-LM	High	Douglas *et al.* 1969
Animalia	*Norwalk virus*	Funabashi	5·9	CIEF	?	Goodridge *et al.* (2004)
Animalia	*Norwalk virus*	Hawaii virus	6·0	CIEF	?	Goodridge *et al.* (2004)
Animalia	*Norwalk virus*	Kashiwa	5·5	CIEF	?	Goodridge *et al.* (2004)
Animalia	*Norwalk virus*	Narita	5·5	CIEF	?	Goodridge *et al.* (2004)
Animalia	*Norwalk virus*	Norwalk virus	5·9	CIEF	?	Goodridge *et al.* (2004)
Animalia	*Norwalk virus*	Seto	6·0	CIEF	?	Goodridge *et al.* (2004)
Animalia	*Papillomavirus*	Papillomavirus	5·0	Aggregation	High	Beard and Wyckoff (1938)
Animalia	*Poliovirus*	PV-1	7·4 and 4·0	IEF-DA	?	Nasser *et al.* (1992)
Animalia	*Poliovirus*	PV-1	6·9	IEF	?	Brioen *et al.* (1985)
Animalia	*Poliovirus*	PV-1 Brunender	7·4 and 3·8	IEF-DA	?	La Colla *et al.* (1972)
Animalia	*Poliovirus*	PV-1 Brunhilde	7·1	IEF-A	?	Zerda and Gerba (1984)
Animalia	*Poliovirus*	PV-1 Brunhilde	7·1 and 4·5	IEF-DA	High	Mandel (1971)
Animalia	*Poliovirus*	PV-1 Chat	7·5 and 4·5	IEF-PA	?	Ward (1978)
Animalia	*Poliovirus*	PV-1 LSc2ab	6·6	IEF-A	?	Zerda and Gerba (1984)
Animalia	*Poliovirus*	PV-1 LSc2ab	6·6	?	?	Murray and Parks (1980)
Animalia	*Poliovirus*	PV-1 LSc2ab	6·75 and 4·1	IEF-DA	Low	Butler *et al.* (1985)
Animalia	*Poliovirus*	PV-1 LSc2ab	6·75 and 4·5	IEF-DA	Low	Butler *et al.* (1985)

Table 1 (*Continued*)

Host kingdom	Virus species	Strain	IEP(s)	Method	Purity	Reference
Animalia	*Poliovirus*	PV-1 Mahoney	8·3	IEF-DA	Low	Floyd and Sharp (1978)
Animalia	*Poliovirus*	PV-2 Sabin T2	6·5 and 4·5	IEF	?	Murray and Parks (1980)
Animalia	*Rotavirus A*	Simian rotavirus A/SA11	8·0	IEF-DA	Low	Butler *et al.* (1985)
Animalia	*Smallpox*	Butler	5·7	IEF-DA	Low	Mouillot and Netter (1977)
Animalia	*Smallpox*	Djibouti	5·6	IEF-DA	Low	Mouillot and Netter (1977)
Animalia	*Smallpox*	Harvey	5·9	IEF-DA	Low	Mouillot and Netter (1977)
Animalia	*Smallpox*	Harvey	3·4	EM-LM	High	Douglas *et al.* (1969)
Animalia	*Smallpox*	Moloya	5·6	IEF-DA	Low	Mouillot and Netter (1977)
Animalia	*Smallpox*	Sidi Amock	5·9	IEF-DA	Low	Mouillot and Netter (1977)
Animalia	*Smallpox*	Teheran	5·6	IEF-DA	Low	Mouillot and Netter (1977)
Animalia	*Smallpox*	Vannes	5·6	IEF-DA	Low	Mouillot and Netter (1977)
Animalia	*Vaccinia*	Chaumier	5·0	IEF-DA	Low	Mouillot and Netter (1977)
Animalia	*Vaccinia*	Connaught	4·9	IEF-DA	Low	Mouillot and Netter (1977)
Animalia	*Vaccinia*	Lister	5·1	IEF-DA	Low	Mouillot and Netter (1977)
Animalia	*Vaccinia*	Lister	3·9	EM-LM	High	Douglas *et al.* (1969)
Animalia	*Vaccinia*	Lister (egg)	3·7	EM-LM	High	Douglas *et al.* (1966)
Animalia	*Vaccinia*	Lister (rabit)	3·0	EM-LM	High	Douglas *et al.* (1966)
Animalia	*Vaccinia*	Rabbitpox (Utrecht)	2·3	EM-LM	High	Douglas *et al.* (1969)
Animalia	*Vaccinia*	WR	4·8	EM-LM	Low	Taylor and Bosmann (1981b)
Animalia	*White cowpox*	Brighton	2·8	EM-LM	High	Douglas *et al.* (1969)
Animalia	*Whitepocks*	64.72.55	5·1	IEF-DA	Low	Mouillot and Netter (1977)
Animalia	*Whitepocks*	64.72.75	4·9	IEF-DA	Low	Mouillot and Netter (1977)
Animalia	*Whitepocks*	Chimp 9	4·8	IEF-DA	Low	Mouillot and Netter (1977)
Animalia	*Whitepocks*	MK7.73	5·3	IEF-DA	Low	Mouillot and Netter (1977)
Animalia	*Whitepocks*	RZ.10.71	5·1	IEF-DA	Low	Mouillot and Netter (1977)
Animalia	*Whitepocks*	RZ.38.75	5·2	IEF-DA	Low	Mouillot and Netter (1977)
Bacteria	*Acholeplasma phage O1*	Acholeplasma phage O1	4·0	?	?	Pawlitschek *et al.* (1962)
Bacteria	*Actinomycetes phage MSP8*	Actinomycetes phage MSP8	3·5	IEF-A	High	Kolstad and Bradley (1966)
Bacteria	*Bacillus phage ϕ29*	Bacillus phage ϕ29	4·2	Moving boundary	Low	Rubio *et al.* (1974)
Bacteria	*Enterobacteria phage BZ13*	Enterobacteria phage GA	2·1, 2·3*	EM-LS	High	Langlet *et al.* (2008b)
Bacteria	*Enterobacteria phage F1*	Enterobacteria phage SP	2·1, 2·6*	EM-LS	High	Langlet *et al.* (2008b)
Bacteria	*Enterobacteria phage MS2*	Enterobacteria phage f2	4·0	IEF-DA	Low	Butler *et al.* (1985)
Bacteria	*Enterobacteria phage MS2*	Enterobacteria phage MS2	3·9	IEF-A	?	Zerda and Gerba (1984)
Bacteria	*Enterobacteria phage MS2*	Enterobacteria phage MS2	3·5	EM-LS	High	Penrod *et al.* (1995)
Bacteria	*Enterobacteria phage MS2*	Enterobacteria phage MS2	3·1, 3·9*	EM-LS	High	Langlet *et al.* (2008b)
Bacteria	*Enterobacteria phage MS2*	Enterobacteria phage MS2	3·9	Moving boundary	High	Overby *et al.* (1966)
Bacteria	*Enterobacteria phage MS2*	Enterobacteria phage MS2	3·9	IEF-DA	?	Nasser *et al.* (1992)
Bacteria	*Enterobacteria phage MS2*	Enterobacteria phage MS2	2·2, 3·3, 3·5*	EM-LS	Low	Yuan *et al.* (2008)
Bacteria	*Enterobacteria phage PRD1*	Enterobacteria phage PR722	3·8–4·2	Chromatofocusing	Low	Brorson *et al.* (2008)
Bacteria	*Enterobacteria phage Qβ*	Enterobacteria phage Qβ	2·7, 1·9*	EM-LS	High	Langlet *et al.* (2008b)
Bacteria	*Enterobacteria phage Qβ*	Enterobacteria phage Qβ	5·3	Moving boundary	High	Overby *et al.* (1966)
Bacteria	*Enterobacteria phage T4*	Enterobacteria phage T2	4·2	Aggregation	?	Sharp *et al.* (1946)
Bacteria	*Enterobacteria phage T4*	Enterobacteria phage T4	2·0	EM-LS	?	Aronino *et al.* (2009)
Bacteria	*Enterobacteria phage T4*	Enterobacteria phage T4	4·0–5·0	IEF-PA	Low	Childs and Birnboim (1975)
Bacteria	*Enterobacteria phage λ*	CI47	3·8	EM-LS	High	Penrod *et al.* (1995)
Bacteria	*Enterobacteria phage μ2*	Enterobacteria phage μ2	4·0	IEF-PA	Low	Piffaretti and Pitton (1976)
Bacteria	*Enterobacteria phage φX174*	Enterobacteria phage S13	7·0	?	High	Aach (1963)
Bacteria	*Enterobacteria phage φX174*	Mutants	7·4	?	High	Aach (1963)
Bacteria	*Enterobacteria phage φX174*	Wild type	6·6	?	High	Aach (1963)
Bacteria	*Enterobacteria phage φX174*	Enterobacteria phage φX174	6·0–7·0	Chromatofocusing	Low	Brorson *et al.* (2008)
Bacteria	*Enterobacteria phage φX174*	Enterobacteria phage φX174	2·6	EM-LS	?	Aronino *et al.* (2009)
Bacteria	*Enterobacteria phage φX174*	Enterobacteria phage φX174	6·6	CIEF	Low	Horká *et al.* (2007)
Bacteria	*Enterobacteria phage φX174*	Enterobacteria phage φX174	6·6	Aggregation	High	Sinsheimer (1959)
Bacteria	*PM 2*	PM 2	7·3	IEF	?	Schaefer *et al.* (1974)
Bacteria	*Pseudomonas phage PP7*	Pseudomonas phage PP7	4·3–4·9	Chromatofocusing	Low	Brorson *et al.* (2008)

Table 1 (*Continued*)

Host kingdom	Virus species	Strain	IEP(s)	Method	Purity	Reference
Plantae	*Belladonna mottle virus*	Belladonna mottle virus	6·3	IEF-A	Low	Petrzik (1993)
Plantae	*Cowpea chlorotic mottle virus*	Cowpea chlorotic mottle virus	3·8	EM-LS	High	Suci *et al.* (2005)
Plantae	*Erysimum latent virus*	Erysimum latent virus	4·7	IEF-A	Low	Petrzik (1993)
Plantae	*Red clover necrotic mosaic virus*	Serotype A	5·0	IEF-A	Low	Gallo and Musil (1984)
Plantae	*Red clover necrotic mosaic virus*	Serotype B	4·8	IEF-A	Low	Gallo and Musil (1984)
Plantae	*Red clover necrotic mosaic virus*	Serotype C	4·6	IEF-A	Low	Gallo and Musil (1984)
Plantae	*Scrophularia mottle virus*	Anagyris	4·4	IEF-A	Low	Honetslegrova *et al.* (1994)
Plantae	*Scrophularia mottle virus*	Czech isolate	3·9	IEF-A	Low	Honetslegrova *et al.* (1994)
Plantae	*Scrophularia mottle virus*	Scrophularia mottle virus	4·0	IEF-A	Low	Petrzik (1993)
Plantae	*Southern bean mosaic virus*	Variant 1	6·0	IEF-DA	High	Magdoff-Fairchild (1967)
Plantae	*Southern bean mosaic virus*	Variant 2	5·6	IEF-DA	High	Magdoff-Fairchild (1967)
Plantae	*Southern bean mosaic virus*	Variant 3	5·0	IEF-DA	High	Magdoff-Fairchild (1967)
Plantae	*Southern bean mosaic virus*	Variant 4	4·0	IEF-DA	High	Magdoff-Fairchild (1967)
Plantae	*Tobacco mosaic virus*	*Cucumber virus 4*	4·9	Aggregation	Low	Oster (1951)
Plantae	*Tobacco mosaic virus*	Green aucuba	4·5	Aggregation	Low	Oster (1951)
Plantae	*Tobacco mosaic virus*	Holmes' masked	3·9	Aggregation	Low	Oster (1951)
Plantae	*Tobacco mosaic virus*	Holmes' rip-gras	4·5	Aggregation	Low	Oster (1951)
Plantae	*Tobacco mosaic virus*	J14D1	4·2	Aggregation	Low	Oster (1951)
Plantae	*Tobacco mosaic virus*	Ordinary	3·9	Aggregation	Low	Oster (1951)
Plantae	*Tobacco mosaic virus*	Yellow aucuba	4·6	Aggregation	Low	Oster (1951)
Plantae	*Turnip yellow mosaic virus*	Turnip yellow mosaic virus	3·6	IEF-A	Low	Petrzik (1993)

IEF, isoelectric focusing; EM, electrophoretic mobility.
IEP fields which show asterisk (*) contain measurements taken under various water chemistry and thus altering IEP value(s). Methods used in the studies were abbreviated as followed: IEF in dense aqueous solutions, e.g. sucrose, ampholine (IEF-DA), IEF in agarose gel (IEF-A), IEF in polyacrylamide gels (IEF-PA), capillary IEF (CIEF), EM using light microscope for detection (EM-LM), EM using laser scattering (EM-LS), and electrical detection using nanowire field effect transistors (EDN-FET). The purity of virus stock is scored to assess possible influence of water chemistry on measurements.

Discussion

The review of 137 IEP measurements resulted in 152 IEP values from 104 viruses. These were listed in Table 1. All IEPs were found to occur in the pH range from 1·9 to 8·4, indicating that viruses with a very basic IEP do not exist. Some viruses have been measured more than once. For example, the *Poliovirus* was measured 12 times with different results. It needs to be noted here that the *Poliovirus* may have two IEPs which leads to a total number of 19 IEP values from these 12 measurements. Within the family of *Picornaviridae*, three species have been found to have two IEPs, first discovered by Mandel 1971. These observations on the *Poliovirus* prompted Mandel to assume that the virion can exist in either of two different but interconvertible states. For the *Encephalomyocarditis virus* (Chlumecka *et al.* 1973) and the *Human enterovirus B* (Murray and Parks 1980; Butler *et al.* 1985), two states were found but were not always confirmed by other studies (see Table 1). In the following paragraphs, we will select some of the viruses IEP values from Table 1 that have been measured more frequently to determine and discuss their variance. To quantify the differences of the IEP measurements, for example within a virus species, we introduce the discrepancy in IEP (ΔIEP) that is simply the subtraction of the lowest reported IEP from the highest one.

Out of Table 1, five IEP values of *Human enterovirus B* were selected. These values were measured within a single study by Zerda and Gerba (1984) in which comparable

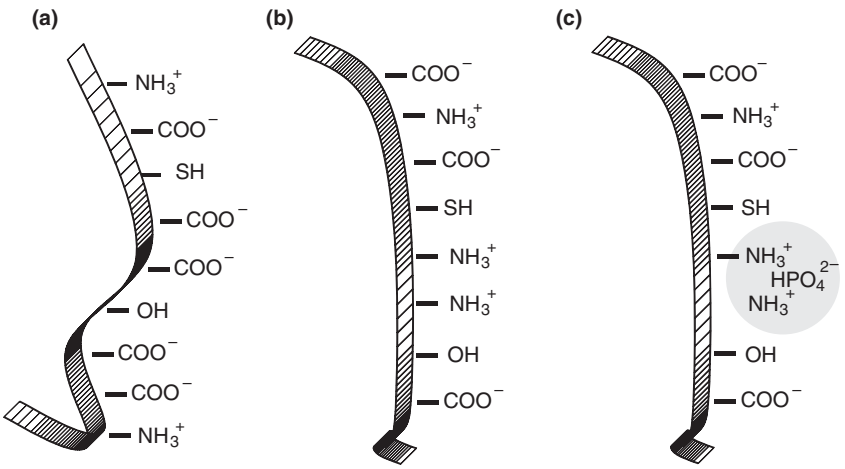

Figure 2 Sketches coat protein segments of different viruses and the arrangement of their functional groups. The environmental pH for all three schematic draws is assumed to be neutral. In (a) and (b), we compare the occurrence of different types of functional groups on two different coat proteins leading 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 which 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).

measurement conditions could be assumed. IEPs were solely distinguished by different strains of the virus resulting in a ΔIEP of 1·4. Magdoff-Fairchild (1967) found a maximum of ΔIEP 2 among four variants of highly purified *Southern bean mosaic virus*, all measured under the same conditions. Other groups confirmed strain dependency within a single study with ΔIEPs of comparable or smaller magnitudes (Aach 1963; Douglas *et al.* 1966; Chlumecka *et al.* 1973; Mouillot and Netter 1977; Gallo and Musil 1984; Honetslegrova *et al.* 1994; Goodridge *et al.* 2004).

Enterobacteria phage MS2 was measured 10 times and shows a mean IEP value of 3·5 with a standard deviation of 0·6 and a ΔIEP of 1·8. Two recent studies by Langlet *et al.* (2008b) and Yuan *et al.* (2008) determined the alteration of the IEP by a change in water chemistry (e.g. ionic strength or ionic composition). Values shifted from 3·1 to 3·9 by increasing the concentration of $NaNO_3$ from 1 to 100 mmol l^{-1}, respectively, and were measured at 2·2, 3·3, and 3·5 by diluting virus stock in phosphate saline buffer, deionised water and $NaHCO_3$, respectively. These changes indicate specific adsorption and/or surface complexation taking place at the virus–water interface as is known for inorganic–water interfaces (Stumm 1992). This is underlined by the study of Hidber *et al.* (1996) which showed that the IEP of alumina (α-Al_2O_3) in water could be altered by the subsequent addition of citric acid to the suspension. Hence, shifting the IEP of pure alumina from pH 9 to pH 3 with a ΔIEP 6. The principal of specific adsorption or surface complexation and its influence on the IEP of the virion are schematically shown when comparing Fig. 2b with 2c. Both schematics have an identical coat protein but do not possess equal net surface charge, as in Fig. 2c some positively charged functional groups became neutralized with a hydrogen phosphate ion. This results in a decrease in positive charges, hence shifting the IEP towards the acid regime. As was shown for the phage MS2 before where the IEP was shifted in the presence of phosphate from 3·3 to 2·2.

The fact that several IEPs were found when electrolyte conditions have been changed does not permit the allocation of the IEP as a virion-specific attribute. If we assume the rather seldom case in which colloidal particles are suspended in absolute pure water, another term is used that, in contrast is characteristic to the virus' surface: the point of zero charge (PZC). However, the nomenclature varies in the literature; for example, the pH at which the net charge of a protein is equal to zero in pure water is called the isoionic point (Righetti and Caravaggio 1976). In the field of geology, it is often termed as the point of zero net charge (Sposito 1998) and so on. In this study, however, we stay with the term PZC, when suspended in pure water and IEP, when suspended in water containing any charged species. This is also reflected in the Fig. 2c,d in which the PZC alters to become the IEP at a different pH

value. Some studies did not distinguish between the IEP and PZC that enhanced the potential of confusion (Parks 1965, Kosmulski 2002). The IEP and the PZC do not necessarily differ in the presence of monovalent ions, and as it is impractical to determine PZC experimentally, we mainly focus on using the term IEP in the current study.

We are now seeking a representative average value of the Enterobacteria phage MS2. Thus, measurements at undefined purity, different strain, and conducted at high ionic strength were excluded to aim towards PZC values. After screening values from Table 1, four IEP values were left: 3·1, 3·3, 3·5, 3·9 resulting in a slight change in the average value from 3·5 to 3·4 but reducing its deviation from 0·6 to 0·3, and ΔIEP from 1·8 to 0·8, hence improving the accuracy. In the case of *Enterobacteria phage ΦX174*, the level of improvement could even be increased. From Table 1, ΦX174 shows the highest ΔIEP of 4·8 (IEP = 6·2 ± 1·6) and after excluding data of different strains and mutants, it was reduced slightly to 4. In this state, it is mainly dependent on one measurement taken by Aronino *et al.* (2009) with an IEP of 2·6. The authors did not mention any purification of virus stock performed prior to measurement, and thus, it was excluded from the average value. The phage now has an IEP of pH 6·6 ± 0·05 with ΔIEP = 0·1. Other studies confirmed the shift in IEP by the water chemistry: Molodkina *et al.* (1986) found that IEP of *Influenza A virus* (H1N1) was altered by concentrations of 0·2, 0·4, 1·5 and 10 mmol l^{-1} NaCl stepwise from 4·5 to 4·35, 4·25 and 4, respectively. Furthermore, Langlet *et al.* (2008b) observed the same effect on three other bacteriophages with a maximum ΔIEP = 0·8. In contrast to these findings, a study on *Mammalian orthoreovirus* by Taylor and Bosmann (1981a) did not show any significant change in the IEP at various NaCl concentrations, although EM decreased with increasing electrolyte concentration as expected from DLVO theory.

Another factor able to alter IEP values might be the host used in laboratory virus multiplication, as shown by Douglas *et al.* (1966). *Vaccinia* (strain: Lister) reproduced in chicken eggs and rabbit cells had different IEPs of 3·7 and 3·0, respectively. In contrast, *Cowpox* (strain: Brighton) showed no alteration following the same procedure in the study. Data merged in Table 1 did not allow the determination of the effect of different measurement techniques on IEP variances, as no values remained constant in all fields (according to scattering impact factors). However, this is no reason to exclude it *per se*. To the author's best knowledge, no study was undertaken to assess the influence of detection methods used for virus IEP determination. However, the detection method is believed to be a potential source of scattering, and thus, further studies would be needed to evaluate this effect. The effect of temperature on the IEP could also not been investigated within this study. Evidence for a potential influence is given by a ΔIEP of 0·5 when proteins have been measured at 4 and 25°C (Righetti and Caravaggio 1976).

Water chemistry, as shown earlier, may influence surface charge in both hard and soft matter. This might be because of specific adsorption of ions, surface complexation, reduction in hydration in the presence of substances such as sucrose, and inner electro osmotic flow within the bio-colloid (Parks 1965; Douglas *et al.* 1966; Langlet *et al.* 2008a,b). Hence, altering the EM and shifting IEPs towards upper or lower pH values. Reported IEP values should thus only be considered in discussions of sorption phenomena if its water chemistry is comparable to the system being discussed. Bacteriophages, in particular *Enterobacteria phages ΦX174* and *MS2*, are frequently used in studies to assess sorption behaviour in aquatic environment. Therefore, the IEP is used for the justification of electrostatic interactions. For example, Dowd *et al.* (1998) found a strong correlation between the adsorption of viruses and their IEPs. The IEPs of the two phages MS2 and ΦX174 have been used to explain their difference in adsorption to sand (You *et al.* 2005). This, however, is in contradiction to the study of Aronino *et al.* (2009) in which only the size of the phages (MS2, ΦX174 and T4) could be correlated to their removal in sand filtration. If such values are indeed wrong, this may lead to incorrect conclusions and underlines the necessity of the present work. The fact that viruses are usually stored in a buffering media containing phosphates or other complexing agents question those IEP measurements in which virus stocks did not undergo any proper purification step prior to measurement.

To display a distribution function of virus' IEPs, it was necessary to find a representative mean value for those virus strains which have been measured more than once. This was carried out according to the procedures applied previously for the bacteriophages MS2 and ΦX174 by excluding certain values and determination of a mean value for the virus strain. If viruses have two IEPs, mean values of either state were accounted to display frequency distribution. Thus, the 152 values could be reduced to 115 that are now found in the pH regime from 2·1 to 8·3, whereas most frequent values appear in the region of 3·5–7. Data are displayed in Fig. 3 as a histogram and fitted with a Gaussian function revealing a mean value of 5·0 and a standard deviation of 1·3.

Conclusions

Virus' IEPs appear in the range 1·9 < IEP < 8·4, following the screening procedure applied in this study The IEP values were found to lie between 2·1 and 8·3 with a mean

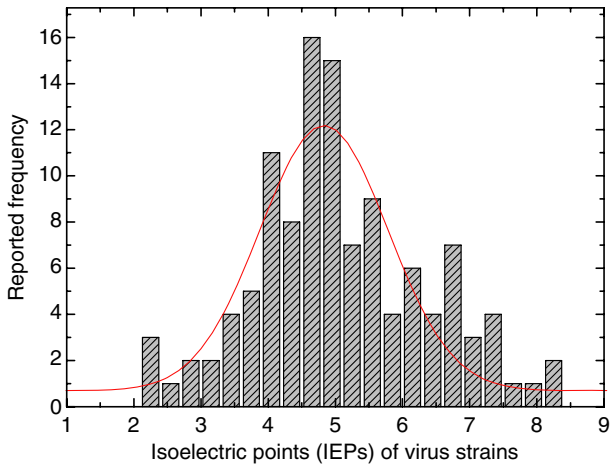

Figure 3 Displays isoelectric points of viruses and their reported frequency in literature. Columns plotted in the range 0 < pH < 10, increment = 0·3. Line presents a Gaussian fit of data resulting in a mean value of 5·0 ± 1·3, R^2 = 0·81.

of 5·0 ± 1·3, indicating that viruses with an IEP in the very basic pH regime do not exist. On the other hand, this could be an artefact as the water chemistry of most virus stocks used in this IEP compilation were either of low or of unknown purity and preferably contain anionic species with high-adsorption capacity (e.g. phosphate and amino acids). A wide variation of data was found among single-virus species what is a result of (i) differentiation in virus strains, assumingly because functional groups in coat proteins vary among strains, (ii) insufficient purification of virus stock leading to the determination of artefacts, (iii) interactions of charged agents with the virus interface such as specific adsorption and/or surface complexation and (iv) diversity in host cells. The influence of methods used for IEP determination could not be assessed within the study but is a potential source of scattering and should be studied in a future work. Care must be taken if IEP values from literature are used to discuss results on virus sorption, as these values are not always identical in water chemistry.

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