Q-433

An Analysis of the Relationship Between Virus Capsid Amino Acid Composition and Removal and Disinfection Efficacy

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Motivation

Why are some viruses removed and inactivated more effectively than others during water and wastewater treatment processes?

Although vast quantities of empirical data exist, the exact mechanisms governing microbial adsorption and inactivation in water and wastewater treatment processes remain unclear.

Does treatability depend on:
- Size?
- Means of infection?
- Genetic composition?
- Genetic arrangement?
- Isoelectic point?

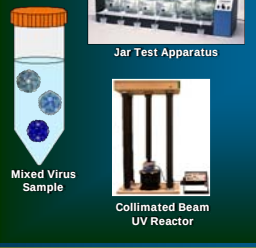
Virus * Theoretical pI based on capsid protein analysis rather than empirical data	MS2	PRD1	phi-X174	fr	Adenovirus 4	Feline calicivirus	Coxsackievirus B6	Echovirus 12	Poliovirus 1 (strain Lsc-2ab)
Infectious host	*E. coli* 15597	*Salmonella typhimurium* LT2	*E. coli* 13706	*E. coli* 19853	PLC/PRF/5	CRFK	BGM	BGM	BGM
Size (nm)	24 – 27	62 – 65	23 – 27	23	70 – 100	27 – 40	27 - 32	27 - 32	27 - 32
Mode of infection	Male-specific	Somatic	Somatic	Male-specific	--	--	--	--	--
Nucleic acid	ssRNA	dsDNA	ssDNA	ssRNA	dsDNA	ssRNA	ssRNA	ssRNA	ssRNA
Topology	Linear	Linear	Circular	Linear	Linear	Linear	Linear	Linear	Linear
Genome length (nt)	3,569	14,927	5,386	3,575	35,994	7,683	7,389	7,389	7,440
GC ratio	52%	48%	44%	51%	57%	45%	47%	47%	46%
Isoelectric point (pI)	3.5 – 3.9	3.0 – 4.2	6.6	8.9 – 9.0	5.15*	4.60*	4.89*	6.23*	7.0 – 8.2
UV dose (4-log) (mJ/cm²)	65	32	10	15	160	32	34	30	31
UV dose reduction with 1 mg/L TiO_2	15%	2.4%	6.3%	0%		--			
Max. removal with 40 mg/L $FeCl_3$ and pH ≤ 6.0	2.7 logs	1.7 logs	1.7 logs	2.5 logs	2.6 logs	2.5 logs	3. 0 logs	1.8 logs	2.5 logs

No single characteristic appears to define treatability, but some demonstrate a stronger relationship than others.

This study examined the effect of virus capsid amino acid composition on viral inactivation using ultraviolet (UV) disinfection, titanium dioxide (TiO_2) photocatalysis, and ferric chloride coagulation.

Experimental Approach

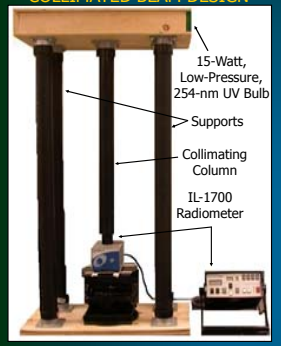
Mixed virus samples were used to conduct UV, TiO_2 photocatalysis, and ferric chloride coagulation jar testing experiments. The resulting samples were assayed using the double agar layer technique or conventional $TCID_{50}$ cell culture for bacteriophages and human/animal viruses, respectively.



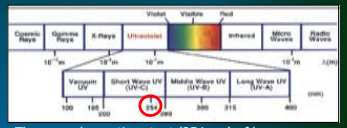
Ultraviolet (UV) Disinfection

The formation of pyrimidine dimers is thought to be the primary mechanism responsible for viral inactivation via UV irradiation. Accordingly, increased UV resistance would be expected for viruses with capsids comprised of high percentages of aromatic amino acids (which are the only amino acids with significant extinction coefficients in the UV-C range of 200 – 280 nm). By absorbing germicidal UV light, these amino acids would reduce UV penetration and damage to the viral nucleic acid.

COLLIMATED BEAM DESIGN

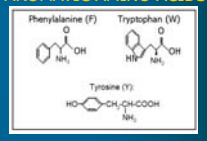


LIGHT SPECTRUM



The monochromatic output (254 nm) of low-pressure UV bulbs is considered the most effective wavelength for disinfection purposes.

AROMATIC AMINO ACIDS



PYRIMIDINE DIMERS

Correction Factors:
Petri = 0.890 Divergence = 0.984
Reflection = 0.975 Water = 0.995

Intensity Calculation:
$I_{WE} = I_0 \times PF \times RF \times DF \times WF = 0.13$ mW/cm²
$I_0 = 0.15$ mW/cm²

Dose Calculation:
Dose = $I_{WE} \times$ Time
Unit = mW-s/cm² = mJ/cm²

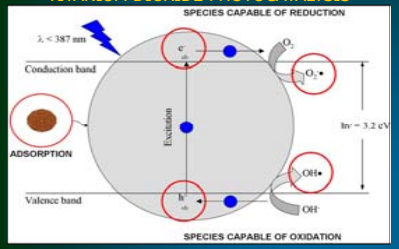
Virus	MS2	PRD1	phi-X174	fr	Ad 4	FCV	Cox B6	Echo 12	Polio 1
Total number of amino acids	23,400	303,040	42,336	23,400	905,220	120,780	39,540	55,980	52,860
Phenylalanine	720 (3%)	12,120 (4%)	2,196 (5%)	900 (4%)	39,996 (4%)	5,940 (5%)	1,800 (5%)	1,800 (3%)	2,220 (4%)
Tryptophan	360 (2%)	2,200 (1%)	516 (1%)	360 (2%)	12,744 (1%)	2,520 (2%)	600 (2%)	1,500 (3%)	780 (1%)
Tyrosine	720 (3%)	17,220 (6%)	1,800 (4%)	720 (3%)	47,820 (5%)	3,780 (3%)	1,560 (4%)	1,800 (3%)	2,340 (4%)
Isoelectric point	3.5 – 3.9	3.0 – 4.2	6.6	8.9 – 9.0	5.15*	4.60*	4.89*	6.23*	7.0 – 8.2
UV dose (4-log) (mJ/cm²)	65	32	10	15	160	32	34	30	31

RESULTS: A correlation between isoelectric point and UV disinfection appears to exist, but possible mechanisms for this are unclear. The analysis did not provide conclusive evidence supporting a correlation between relative composition or absolute quantities of aromatic amino acids and UV inactivation.

Titanium Dioxide (TiO_2) Photocatalysis

Advanced oxidation processes (AOPs) such as TiO_2 photocatalysis rely on the disinfection capabilities of highly reactive radical species. Proteins are more susceptible to radical-induced cleavage at specific amino acids, particularly at proline, glycine, and alanine. Therefore, viruses with high levels of these amino acids in their protein capsids may be more susceptible to photocatalysis.

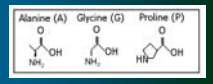
TITANIUM DIOXIDE PHOTOCATALYSIS



TITANIUM DIOXIDE

- Degussa P25 TiO_2 = 75% Anatase and 25% Rutile
- Individual particle size ≈ 25 nm
- Particle aggregation ≈ 500 nm

POSSIBLE HYDROXYL RADICAL AMINO ACID TARGETS



EFFECT OF PROTEIN CAPSID COMPOSITION ON PHOTOCATALYTIC INACTIVATION

Bacteriophage	PRD1		phi-X174		fr		MS2	
Spiking level (PFU/mL)	7.97×10^8		1.16×10^9		9.70×10^8		8.07×10^8	
Total number of amino acids[a]	303,040		42,336		23,400		23,400	
Alanine (A)	34,800	2.70^b	2,952	1.50	2,880	1.73	2,520	1.19
Glycine (G)	19,100	1.48	3,192	1.63	1,620	0.97	1,620	0.76
Proline (P)	14,900	1.16	2,508	1.28	900	0.54	1,080	0.51
Level of inactivation in the Photo-Cat	2.61 logs[c]		2.36 logs		1.99 logs		1.80 logs	

[a]All amino acid values reported per viral particle.
[b]Hypothetical surface density reported in residues/nm².
[c]Pilot-scale photocatalytic inactivation: TiO_2 = 400 mg/L and energy input of 34 mJ/cm².

Hawkins and Davies (1998) and Jones et al. (2007) reported OH• specificity with alanine, glycine, and proline residues. The protein capsid compositions of the bacteriophages supported these theories.

PURIFICS® PHOTO-CAT LAB®

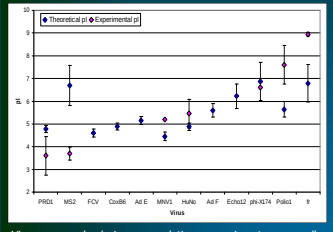
RESULTS: The amino acid capsid compositions of the bacteriophages MS2, PRD1, phi-X174, and fr appear to correlate with photocatalytic inactivation on the basis of absolute amino acid quantity and hypothetical surface density. For example, PRD1 experiences the highest level of photocatalytic inactivation and also contains the most alanine, glycine, and proline residues. Other factors may play a significant role in photocatalytic inactivation, but the data seems to correlate well with the presence of hydroxyl radical amino acid targets in the capsid.

Ferric Chloride ($FeCl_3$) Coagulation

The isoelectric point (pI) of a viral particle, which depends on the amino acid composition of the protein capsid, is believed to strongly influence viral adsorption in soil and water. The relationship between pI and fractional amino acid composition of virus capsids was examined to determine the potential effect on viral behavior and stability in water.

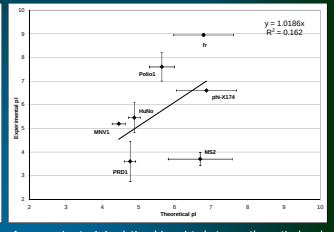
Amino acids that dictate pI

VIRUS ISOELECTRIC POINTS



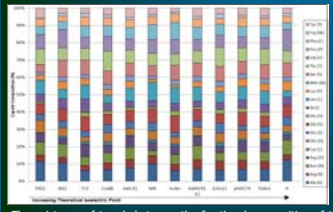
Virus removal during coagulation experiments generally reflected the trend shown for the above pIs. For example, qualitative bacteriophage removal was generally PRD1 < MS2 < phi-X174 < fr, which follows the pI trend.

RELATIONSHIP BETWEEN THEORETICAL AND EMPIRICAL ISOELECTRIC POINTS

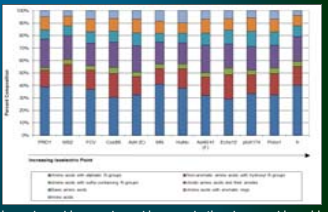


An approximate 1:1 relationship exists between theoretical and empirical pIs. Theoretical pI calculations may be less accurate for viruses with empirical pIs toward the extremes (e.g., MS2 and fr), perhaps since the calculation doesn't account for chemical modifications.

VIRUS CAPSID AMINO ACID COMPOSITION



VIRUS CAPSID AMINO ACID GROUP COMPOSITION



The existence of trends between the fractional composition of the amino acids or amino acid groups in the virus capsid would contribute to an improved understanding of the affect that capsid composition has on viral particles and their behavior/stability in water. As shown in the above figures, no such trends are readily apparent.

RESULTS: Physical removal of viruses by adsorption during coagulation was strongly correlated to pI ($-0.5 \leq$ Pearson Coefficient ≤ 0.5), but not to virus size. The analysis did not identify a strong relationship between capsid amino acid composition, pI, and adsorption. Additional analyses of the affects of protein folding and surface exposure on pI and adsorption are recommended.

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