Supporting Information

Heteroaggregation of Virions and Microplastics Reduces the Number of Active Bacteriophages in Aqueous Environments

Enkhlin Ochirbat [1, ‡], Rafał Zbonikowski [1, ‡], Anna Sulicka [1,2], Bartłomiej Bończak [1], Magdalena Bonarowska [1], Marcin Łoś [3,4], Elżbieta Malinowska [2,5], Robert Hołyst [1], Jan Paczesny [1,*]

eochirbat@ichf.edu.pl, rzbonikowski@ichf.edu.pl, anna.sulicka@o2.pl, bbonczak@ichf.edu.pl, mbonarowska@ichf.edu.pl, mlos@biotech.ug.gda.pl, elzbieta.malinowska@pw.edu.pl, rholyst@ichf.edu.pl, jpaczesny@ichf.edu.pl

Affiliations: [1] Institute of Physical Chemistry, Polish Academy of Sciences, Kasprzaka 44/52, 01-224 Warsaw, Poland

[2] Warsaw University of Technology, Faculty of Chemistry, The Chair of Medical Biotechnology, Noakowskiego 3, 00-664, Warsaw, Poland

[3] Department of Molecular Biology, University of Gdansk, Wita Stwosza 59, 80-308 Gdansk, Poland

[4] Phage Consultants, Partyzantów 10/18, 80-254 Gdansk, Poland

[5] Warsaw University of Technology, CEZAMAT, Poleczki 19, 02-822, Warsaw, Poland

*** Corresponding Author:** Jan Paczesny jpaczesny@ichf.edu.pl, +48 22 343 2071

1. Materials and Methods

1.1. Chemicals

LB-agar contained 15 g/L of agar, 10 g/L of NaCl, 10 g/L of tryptone, and 5 g/L of yeast extract and was used as an instant mix (Carl Roth, Germany). LB-medium had the same composition except for the lack of 15 g/L of agar (Carl Roth, Germany). TM buffer (pH=7.4) was prepared using 10 mM Tris base, 5 μM $CaCl_2$, 10 mM $MgSO_4$, and ultrapure water. All chemicals were purchased from Sigma Aldrich (Germany). All solutions were sterilized by autoclaving before use.

1.1. Preparation of the bacteriophages

Phages were obtained from Phage Consultants, Partyzantów, Gdańsk, Poland. An early logarithmic culture of *Escherichia coli* BL21 was infected by T4. For MS2 and M13 multiplication, the *E. coli* C3000 strain was used. After lysis, T4 and M13 phages were precipitated using polyethylene glycol. The precipitates of phages T4 and M13 were purified by centrifugation and diluted with 1 M NaCl. Then CsCl gradient centrifugation was applied (Beckman Optima XL70 ultracentrifuge, Ti50 rotor, 100000 g). T4 and M13 suspensions were dialyzed against a series of TM buffers of decreasing ionic strength. Afterward, 0.2 μg/mL Viscolase (A&A Biotechnology) was added to samples with phages T4 and M13 to digest residual DNA remaining in TM buffer after the procedure. In the case of MS2, the lysate was only filtered using 0.22 μm syringe filters.

1.2. Evaluation of the number of active phages in the suspensions – double overlay method

A double overlay method and droplet plaque counting test were conducted to assess phage activity and virulence. A solution containing 0.4 mL LB medium and 0.5% agar was mixed with 200 μl of refreshed *E.coli* bacteria. Depending on the phage type, they were *E. coli* BL21 (T4) or *E. coli* C3000 (MS2 and M13). The solution prepared in this way was poured onto a

previously prepared petri dish with LB-agar (LB medium and 1.5% agar). After the agar with the bacteria solidified, at least eight droplets (5 µl each) of each adequately diluted phage suspension were deposited onto the plate. Subsequently, the plates were incubated at 37 °C for 24 h. After removing the plates from the incubator, the plaques were counted, and the concentration of phages was calculated and expressed in PFU/mL (plaque-forming units).

Microplastic and leachable experiments were done in triplicates. At least eight technical replicates (droplets) were used for each experimental run to calculate phage titers. Standard deviation is used as an experimental error. The Student`s t-test was performed to evaluate whether the observed differences were statistically significant. * p-value < 0.05; ** p-value < 0.01; *** p-value < 0.001.

Table S1. Comparison of physical characteristics of MS2, M13, and T4 bacteriophages.

	MS2	**M13**	**T4**
shape	icosahedral	filamentous	complex (tailed)
size	23 to 28 nm (Kuzmanovic et al., 2003)	880 x 5.5 nm (Moghimian et al., 2016)	115 x 85 nm capsid, 92 x 24 nm tail, 145 nm motile fibers (Leiman et al., 2010; Yap and Rossmann, 2014)
genetic material	ssRNA	ssDNA	dsDNA
genome size	3569 nucleotides	6407 nucleotides	168 903 bp
zeta potential at pH around 7	around -40 mV (Armanious et al., 2016)	-18 mV (Putra et al., 2019)	-26 mV (Hosseinidoust et al., 2011)
dipole moment	NA	NA	24 kD (200 kD when the fibers are extended) (Greve and Blok, 1975)

1.4. Microplastic BET characterization

Surface areas, total pore volumes, and average pore size of polymers were determined by Micromeritics ASAP2020. The weight of the samples was ~0.3 g. Before measurements, the samples were degassed in a vacuum at 343 K for 5 hours to clean their surface. The adsorption process was carried out at a temperature of liquid nitrogen (77 K), and krypton was used as an adsorbent instead of the commonly used nitrogen. For extremely low surface area samples (which is what the polymers discussed in this paper are), the number of non-adsorbed gas molecules at adsorption equilibrium can exceed the number of molecules adsorbed on the sample, which will hamper the sample's accurate measurement of gas uptake. Because of this effect, the typical surface area detection limit for N_2 physisorption at 77 K is assumed to be about 1 m^2. This detection limit may be significantly reduced by using krypton adsorption analysis at the same temperature that krypton is below its triple point and where its saturation pressure is 2.32 mbar, i.e., ~430 times lower than p_{sat} of N_2. It follows that at any given relative gas pressure, the absolute pressure of Kr is 430 times lower than that of N_2. This also means that the density of Kr in the free space is proportionally lower, which leads to a significant improvement in the detection limit for Kr. The surface area calculation was carried out following the theory of Brunauer, Emmet, Teller (BET) as recommended by the IUPAC for low surface substances (Thommes et al., 2015). The BET model was applied in the range of p/p_0 from 0.05 to 0.30, and the resulting BET plots showed linearity (the measurements error level was 5%).

1.5. Microplastic size analysis based on optical microscopy pictures

Radii of the plastic microparticles based on optical microscopy were calculated with ImageJ 1.53t. Firstly, the pictures of microparticles were adjusted to have sufficient contrast with the background, and any imperfections like light reflexes on the particle surface were masked manually. Then, the areas of the plane projections were integrated, and the radii of the

corresponding circles of the same area were calculated. Data is presented in **Table S2** and in **Figure S1**.

Table S2. Sizes of particles calculated from images taken with a microscope (#1) and calculated results from the BET measurement (#2).

Polymer	**Radius (µm) #1 microscope**	**Radius (µm) #2 BET**
ABS	19.12 ± 10.42	32.6
HIPS	24.80 ± 11.86	30.7
PA6	31.57 ± 10.92	51.6
PC	9.60 ± 8.55	7.8
PE	29.09 ± 10.80	21.1
PET	17.67 ± 5.20	22.2
PMMA	2.35 ± 1.65	2.7
PTFE	36.72 ± 6.17	47.0
PP	36.75 ± 6.16	36.6
PS	42.41 ± 6.20	38.9
PUR	25.15 ± 8.34	17.2
PVC	29.76 ± 7.05	22.6

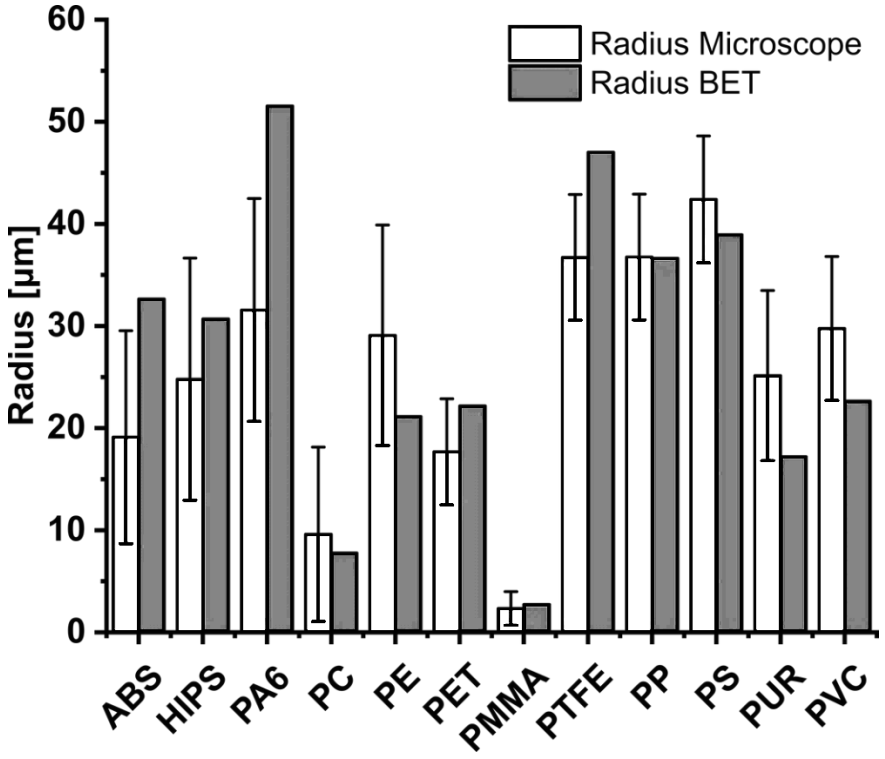


Fig. S1. Comparison of calculated radii size between direct measurement *via* microscope and BET measurement.

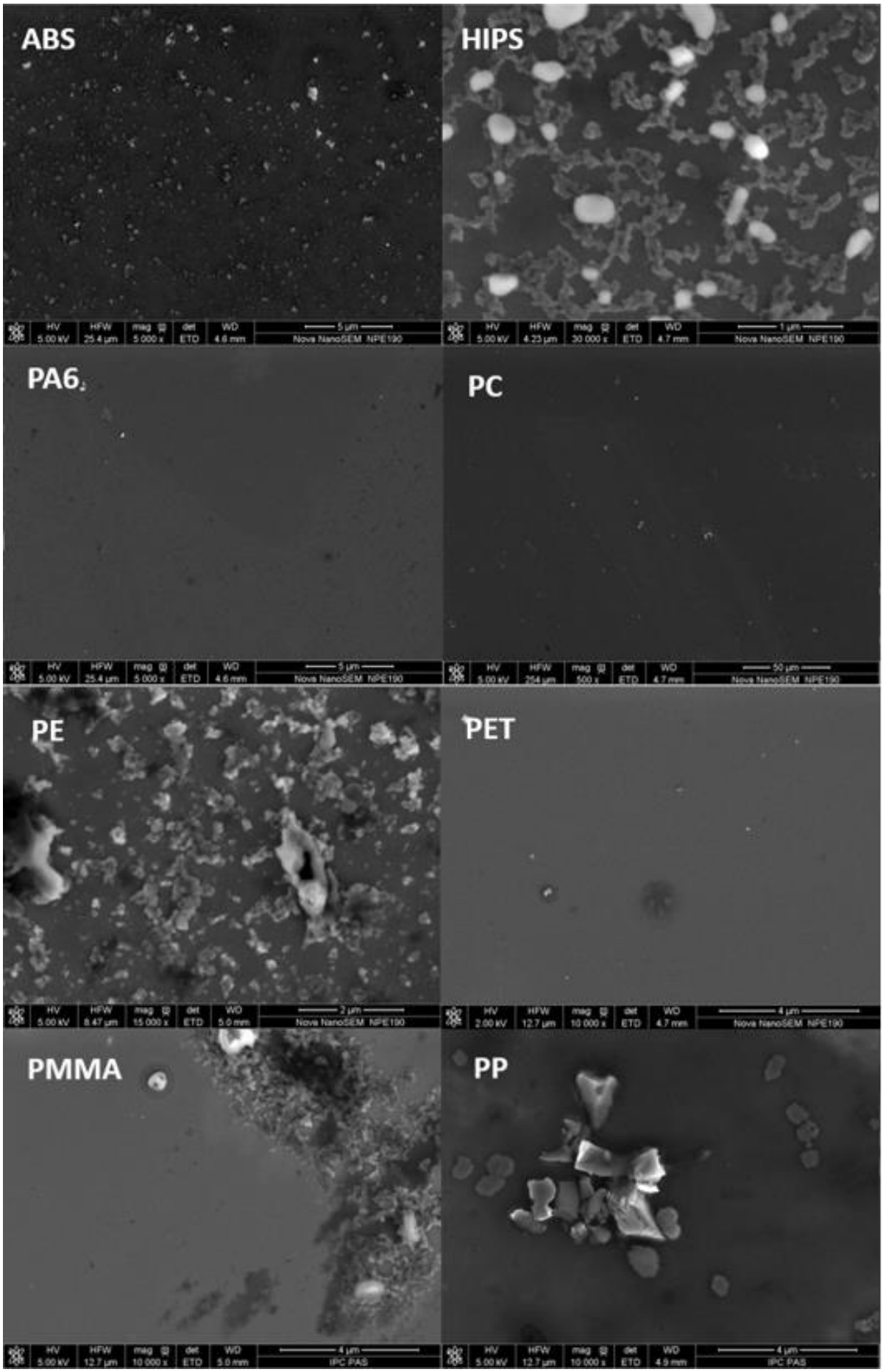
1.6. Total organic carbon (TOC) measurement

We prepared 40 mg samples of each polymer and soaked them in 40 mL of Milli-Q water for 24 h (mixing). After filtration with a syringe filter (PES, 0.22 µm), resulted extracts were analyzed using Elementar – Vario EL III, CHNS to obtain total organic carbon. The results from all of the samples were below the detection limit (<0.03%) of the utilized instrument. Therefore, the analyzes were outsourced to a commercial and accredited laboratory (GBA POLSKA LLC). The measurements were done following the norm (PN-EN 1484:1999) with a lower detection limit (2 ppm). Only two samples, PA6 (4.5 ± 0.7 ppm) and PUR (3.5 ± 0.5 ppm), gave results above the detection limit.

1.7. SEM pictures taken from leachable samples.

SEM pictures were taken (FEI Nova NanoSEM 450) to identify the sizes of particles solubilized in the leachable samples. We applied the leachables'suspentions on polished silica plates and

allowed them to dry out. A minimal number of objects were observed in PC, PET, PA6, PTFE, and PVC cases. We found a moderate number of particles in the case of HIPS and PMMA. For ABS, PP, PE, and PS samples, substantial quantities of particles equally distributed over the silica plate were found. The SEM pictures shown in **Figure S2** showed that, at least in some cases, microplastic fragmented further during the experiment into nano- and sub-microparticles.



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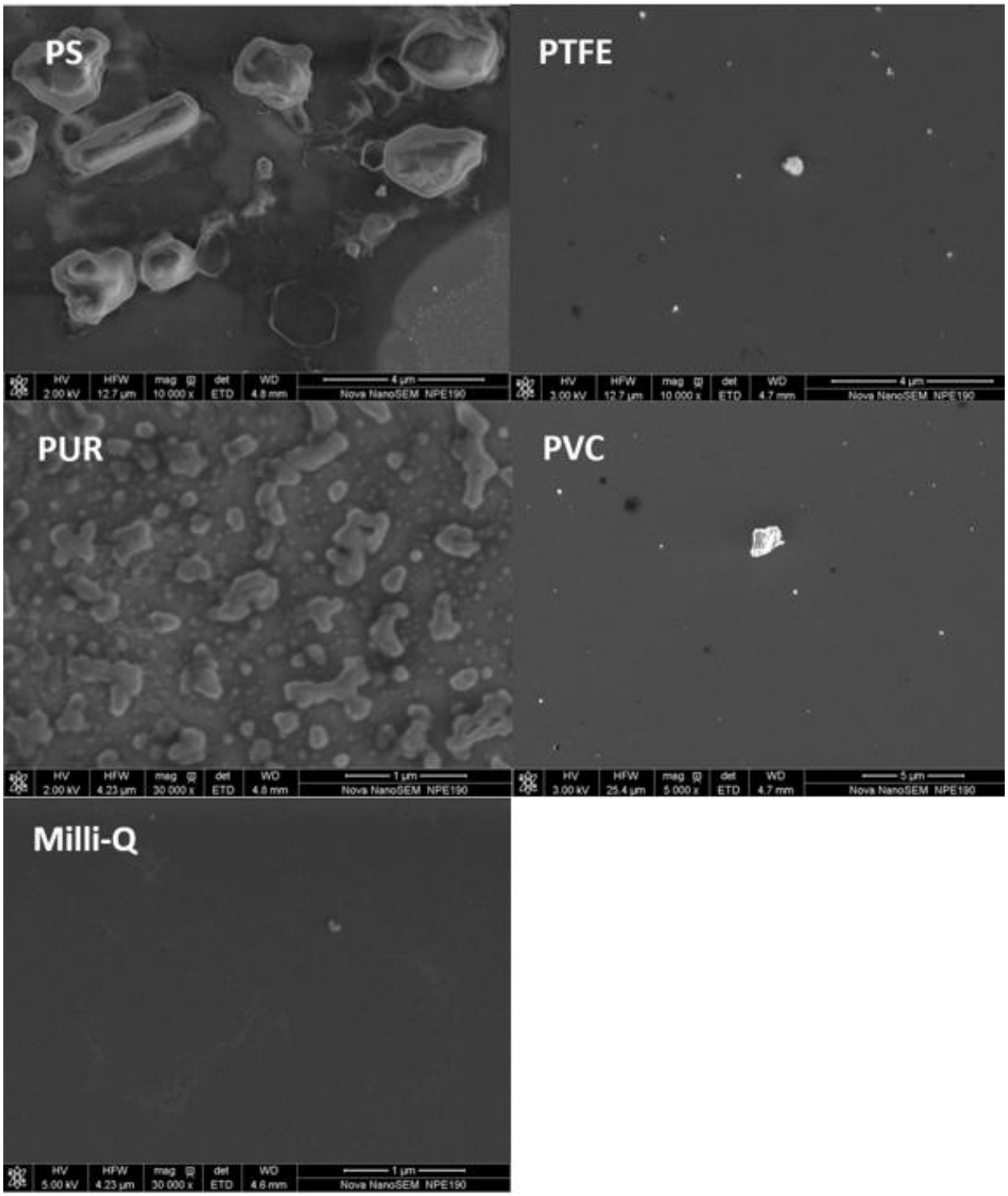

Figure S2. SEM images demonstrating leachable and/or nanoparticles derived from filtered samples incubated with microplastics.

2. Analysis of the results using the Classical Linear Regression Model (Mycielski, 2010)

The **database** used for the analysis is provided as a separate file in the Supporting Information (accudynetest.com access: 12.04.22; polymerdatabase.com access: 12.04.22; Kolska et al. 2013);(Hosseinidoust et al., 2011; Armanious et al., 2016; Putra et al., 2019).

We adjusted the numbering of the equations in the Supporting information to match the main text.

The dependent variable y_i was reserved for the percentage rate of phage titer after 24 h, and taken as the mean value of three repetitions (*cf.* **Table 1** in the main text). To facilitate the interpretation and increase the quality of the regression, an additional observation was added to the database - $y_0 = 100\%$ for all the explanatory variables equal to 0.

At the initial state of modeling, we compared the distribution of y and $\ln y$ with the fitted normal distribution (bins = 10) (**Figure S3**). Based on the observations and a few initial regressions, we decided not to use a logarithmic variable for further analysis.

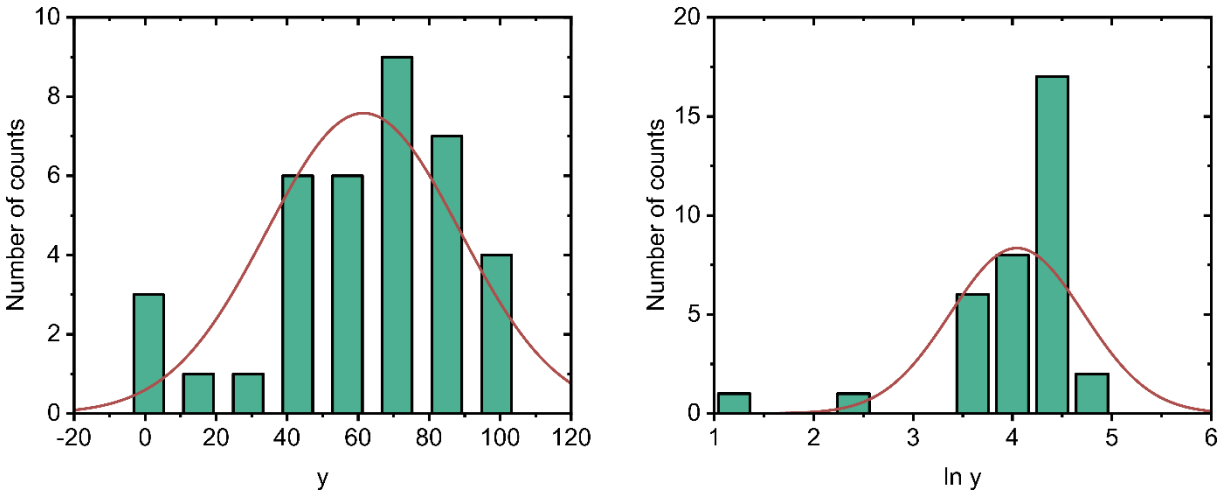

Figure S3. Histograms of the possible functional form of the explained variable and its normal distribution fit (left – y, right – ln y).

Among the explanatory variables were:

- Zeta potential of the polymer (*zeta*), the contact angle of the polymer found in the literature (*conangle*), the measured wetting angle (*wetangle*), the results of the BET measurement (*bet*), the density of the polymer (*density*), the influence of the leachables measured as a percentage drop from **Table 1** (main text) (*leachable*);
- Functions of continuous variables, for instance, *coswetangle* = cos(*wetangle*), *zeta2* = $zeta^2$, tanh(a *zeta*), where a is a parameter between 0.00001 to 0.100000;
- Categorical variables concerning bacteriophage like (*t4, ms2, m13*), used polymer (*abs, hips, pa6, pc, pe, pet, pmma, pp, ps, ptfe, pur, pvc*);
- Binary variables like *color* (if the color of the polymer is not white, indicating the addition of specific dyes), *hydrophob* (if the wetting angle of the polymer was higher than 90 °);
- Interactions of variables and functions of those interactions, for example, *int_t4_zeta = t4·zeta* presenting the value of zeta potential of the polymer if the used phage was T4, unless it returns 0.

All the used variables with the description can be found in the database.

2.1. Coarse estimation

The coarse estimation for categorical variables was conducted. The variables describing the bacteriophage and the polymer were divided into binary variables *(ms2, m13, abs, hips, pa6, pc, pe, pet, pmma, pp, ps, ptfe, pur, pvc)*. Variable *pp* = 1 was taken as the base level.

Source	SS	df	MS	Number of obs	=	37
				F(14, 22)	=	6.43
Model	21079.1708	14	1505.65506	Prob > F	=	0.0001
Residual	5154.40675	22	234.291216	R-squared	=	0.8035
				Adj R-squared	=	0.6785
Total	26233.5775	36	728.710487	Root MSE	=	15.307

y	Coefficient	Std. err.	t	P>|t|	[95% conf.	interval]
t4	-25.59583	18.03897	-1.42	0.170	-63.00637	11.8147
ms2	-32.055	18.03897	-1.78	0.089	-69.46554	5.355536
m13	-45.50917	18.03897	-2.52	0.019	-82.9197	-8.098631
abs	-17.65333	12.49777	-1.41	0.172	-43.57211	8.265446
hips	6.949999	12.49777	0.56	0.584	-18.96878	32.86878
pa6	11.87	12.49777	0.95	0.353	-14.04878	37.78878
pc	13.19	12.49777	1.06	0.303	-12.72878	39.10878
pe	13.59667	12.49777	1.09	0.288	-12.32211	39.51545
pet	-50.42	12.49777	-4.03	0.001	-76.33878	-24.50122
pmma	12.66	12.49777	1.01	0.322	-13.25878	38.57878
pp	0	(omitted)				
ps	-20.79	12.49777	-1.66	0.110	-46.70878	5.12878
ptfe	14.92333	12.49777	1.19	0.245	-10.99545	40.84211
pur	-10.27333	12.49777	-0.82	0.420	-36.19211	15.64545
pvc	-41.71333	12.49777	-3.34	0.003	-67.63211	-15.79455
_cons	100	15.30657	6.53	0.000	68.25611	131.7439

The estimation showed that separate effects of replacing a particular polymer and choosing the bacteriophage could describe the variance of the investigated phenomenon in almost 80%. However, the estimated parameters had a relatively high standard error.

The regression with only the type of the polymer (and not phage type) revealed the average decrease in the activity of phages due to a specific polymer. R^2 dropped from around 0.80 (polymers and phages) to around 0.71 (only polymers).

Source	SS	df	MS	Number of obs	=	37
				F(12, 24)	=	4.88
Model	18602.0657	12	1550.17214	Prob > F	=	0.0005
Residual	7631.51184	24	317.97966	R-squared	=	0.7091
				Adj R-squared	=	0.5636
Total	26233.5775	36	728.710487	Root MSE	=	17.832

y	Coefficient	Std. err.	t	P>|t|	[95% conf.	interval]
abs	-52.04	20.5906	-2.53	0.018	-94.53691	-9.543086
hips	-27.43667	20.5906	-1.33	0.195	-69.93358	15.06025
pa6	-22.51667	20.5906	-1.09	0.285	-65.01358	19.98025
pc	-21.19667	20.5906	-1.03	0.314	-63.69358	21.30025
pe	-20.79	20.5906	-1.01	0.323	-63.28691	21.70691
pet	-84.80667	20.5906	-4.12	0.000	-127.3036	-42.30975
pmma	-21.72667	20.5906	-1.06	0.302	-64.22358	20.77025
pp	-34.38667	20.5906	-1.67	0.108	-76.88358	8.110247
ps	-55.17667	20.5906	-2.68	0.013	-97.67358	-12.67975
ptfe	-19.46333	20.5906	-0.95	0.354	-61.96025	23.03358
pur	-44.66	20.5906	-2.17	0.040	-87.15691	-2.163087
pvc	-76.1	20.5906	-3.70	0.001	-118.5969	-33.60309
_cons	100	17.83198	5.61	0.000	63.19659	136.8034

2.2. Building the model

The standard protocol is to start building a model by eliminating irrelevant variables. However, in our case, having a limited number of observations, we decided to do it from the bottom by trial and error. We replaced categorical variables with physicochemical variables related to adsorption, thermodynamics, heteroaggregation, and the physical chemistry of colloids.

The raw data revealed a relatively weak linear dependence of *y* on *zeta*.

Source	SS	df	MS	Number of obs	=	37
				F(1, 35)	=	4.60
Model	3048.96832	1	3048.96832	Prob > F	=	0.0389
Residual	23184.6092	35	662.417406	R-squared	=	0.1162
				Adj R-squared	=	0.0910
Total	26233.5775	36	728.710487	Root MSE	=	25.737

y	Coefficient	Std. err.	t	P>|t|	[95% conf.	interval]
zeta	.6259195	.291748	2.15	0.039	.0336396	1.218199
_cons	91.17702	14.66314	6.22	0.000	61.40927	120.9448

According to the DLVO theory (Derjaguin et al., 1987; Ohshima, 2012), describing the stability of identical spheres (colloid), interaction energy can be defined as a sum of the van der

Waals attraction energy and double layer repulsion energy. Repulsion free energy of two spheres can be described as:

$$E = \frac{64\pi k_B T R \rho_\infty \gamma^2}{\kappa^2}\, e^{-\kappa d} \quad (3)$$

$$\gamma = \tanh\left(\frac{z e_0 \psi_0}{4 k_B T}\right) \quad (4)$$

where, R – radius of the sphere, ρ_∞- the number density of ions in the bulk solution, γ – reduced surface potential, d – the distance between spheres, z – valency of the ion. We assumed that based on this theory, we have to sort the *zeta* variable and couple it with the bacteriophage type. Various phages have very different physicochemical properties (*cf.* **Table S2**). Thus, we introduced interactions between the phage type and the polymer's zeta potential. Then, by the simple estimation, we proposed that the reduced surface potential should depend on zeta potential ζ as described:

$$\gamma = \tanh(a\zeta) \quad (7)$$

$$a \in [0.00001, 0.10000]$$

Because the energy of the repulsion depends on γ^2 (equation 3) we assumed that the best linear regression was reached (the highest R^2) for the lowest a, when the R^2 reached the same value as for the squared interaction of polymer zeta potential and the phage type. Hence, to simplify the model, we decided to include interactions of phage and squared zeta potential of the polymer (*int_t4_zeta2*, *int_ms2_zeta2*, *int_m13_zeta2*).

Source	SS	df	MS	Number of obs	=	37
				F(3, 33)	=	3.72
Model	6630.62157	3	2210.20719	Prob > F	=	0.0208
Residual	19602.956	33	594.028969	R-squared	=	0.2528
				Adj R-squared	=	0.1848
Total	26233.5775	36	728.710487	Root MSE	=	24.373

y	Coefficient	Std. err.	t	P>|t|	[95% conf.	interval]
int_t4_zeta2	-.0020935	.0031583	-0.66	0.512	-.0085191	.0043321
int_ms2_zeta2	-.0058698	.0031583	-1.86	0.072	-.0122954	.0005558
int_m13_zeta2	-.009881	.0031583	-3.13	0.004	-.0163066	-.0034554
_cons	76.08184	7.52908	10.11	0.000	60.76381	91.39986

The variable *int_t4_zeta2* was excluded from the further analysis as the value of the coefficient oscillated around 0 with a high standard error. P-value showed that we could not say if this parameter is not equal to zero on any rational significance level (neither 0.05 nor 0.10).

We assumed the hydrophobicity of the polymer as an essential variable describing the phenomenon. However, we did not get satisfying results by introducing variables like wetting angle, trigonometrical functions of wetting angle, contact angle, binary segregation of polymer based on the condition θ>90 °, and other derivatives. We concluded that a better variable might be related to the work of adhesion (the work needed to separate a unit of area of two phases) described by the Young-Dupré equation, where σ – surface tension of the liquid (Schrader, 1995):

$$W = \sigma(1 + \cos\theta) \quad (8)$$

The total work is proportional to the area. Therefore, we introduced the new variable

$$betcoswet = (bet + bet\cos(wetangle) \quad (9)$$

This still did not improve the model. Similar variables (for instance, $langmuircoswet$), which were tested, are described in the database.

Further analysis revealed significant improvements in the model quality by using the *radius* being a calculated radius of an average plastic particle according to the equation:

$$r\ (\mu m) = \frac{3}{bet \cdot density} \quad (6)$$

The best outcomes were found when *radius* and *radius2* (*radius*^{2}) were used simultaneously (i.e., the dependence on r was nonlinear). Other similar variables which were tested are described in the database.

These preliminary findings brought us to a few potential models depending on the approach towards the analysis: **Model 1A** and **Model 1B**, and **Model 2.**

Model 1A and **1B** are entirely based on physicochemical data. This analysis omitted PUR, because this polymer might be produced with different monomers and additives. Literature data for PUR varies so much that we decided to omit the polymer from the model.

Model 2 was created by looking for a physicochemical parameter that could describe most of the results of the experiments. The other physicochemical properties were included in categorical variables of a particular polymer and removed from the regression if their impact was not significant (significance level 0.05). This approach assumed that few parameters would govern the phenomenon and are not neglectable. At the same time, modeling allowed for some polymers to have specific features, which caused inconsistency with the rest of the results.

2.3. Model 1A

This approach combined variables related to zeta potential squared concerning the specific phage, the radius of the particles in nonlinear form ($\beta_i r + \beta_j r^2$) and cosine of contact angle.

Source	SS	df	MS	Number of obs	=	34
				F(6, 27)	=	8.53
Model	16969.1009	6	2828.18349	Prob > F	=	0.0000
Residual	8948.00001	27	331.407408	R-squared	=	0.6547
				Adj R-squared	=	0.5780
Total	25917.101	33	785.366696	Root MSE	=	18.205

y	Coefficient	Std. err.	t	P>|t|	[95% conf.	interval]
int_t4_zeta2	-.0009271	.0025219	-0.37	0.716	-.0061016	.0042475
int_ms2_zeta2	-.0045919	.0025219	-1.82	0.080	-.0097664	.0005827
int_m13_zeta2	-.0087035	.0025219	-3.45	0.002	-.013878	-.0035289
radius	-3.368492	.7750578	-4.35	0.000	-4.958779	-1.778205
radius2	.0520132	.0142139	3.66	0.001	.0228487	.0811776
cosconangle	-81.8548	19.71571	-4.15	0.000	-122.3081	-41.40149
_cons	119.0144	10.8045	11.02	0.000	96.84541	141.1834

F-statistic, R^2, adjusted R^2

As the F-statistic is equal to 8.53 and the p-value ≈ 0.0000, we can conclude that there is a statistically significant relationship between y and the chosen explanatory variables. 65.47% of the variance of y is explained by the selected explanatory variables. The adjusted R^2 (correcting the positive bias related to the number of observations and number of explanatory variables) shows that the model should explain 57.80% of the variance of y in the population.

Ramsey RESET test

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Ramsey RESET test for omitted variables
Omitted: Powers of fitted values of y

H0: Model has no omitted variables

F(3, 24) =   3.30
Prob > F = 0.0377
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With the p-value of 0.0377, we reject the hypothesis of the correct specification of the model (significance level of 0.05). However, in our analysis, we suspected that the model's linear form might not be sufficient when all qualitative (categorical or binary) variables were excluded from

the model. A similar model, neglecting *int_t4_zeta2* and *int_t4_zeta2*, passed the RESET test. Nonetheless, we decided to keep those variables.

On the significance level of 0.05, we failed to reject the hypothesis that the estimated parameters of variables *int_m13_zeta2*, *cosconangle*, *radius,* and *radius2* are equal to zero. For *int_ms2_zeta2* we fail to reject the same hypothesis on the significance level = 0.10. *int_t4_zeta2* was kept in the model for the reason described in the previous paragraph.

Correlation Matrix

As suspected, there is high collinearity between variables *radius* and *radius2*, but not other variables.

	int_t4~2	int_ms~2	int_m1~2	radius	radius2	coscon~e
int_t4_zeta2	1.0000					
int_ms2_ze~2	-0.3042	1.0000				
int_m13_ze~2	-0.3042	-0.3042	1.0000			
radius	-0.0188	-0.0188	-0.0188	1.0000		
radius2	-0.0555	-0.0555	-0.0555	0.9577	1.0000	
cosconangle	-0.0447	-0.0447	-0.0447	-0.5215	-0.4895	1.0000

Multicollinearity

Again, as expected *radius* and *radius2* present high VIF values, but they were introduced to the model on purpose. VIF values for other variables are much lower than 10.

Variable	VIF	1/VIF
radius2	13.91	0.071878
radius	13.90	0.071924
int_m13_ze~2	1.52	0.656327
int_ms2_ze~2	1.52	0.656327
int_t4_zeta2	1.52	0.656327
cosconangle	1.42	0.704801
Mean VIF	5.63	

Residuals analysis and homoskedasticity

According to the Shapiro-Wilk test, we fail to reject the 0-hypothesis of the normal distribution of residuals in the model at the significance level of 0.05 (p-value = 0.26195). The distribution is presented in **Figure S4**. The Kernel density plot (normalized to the number of counts) almost

matches the Gaussian curve. There was no strong skewness, nor was kurtosis noticed (too few observations may cause it).

Shapiro-Wilk W test for normal data

Variable	Obs	W	V	z	Prob>z
e	34	0.96111	1.358	0.637	0.26195

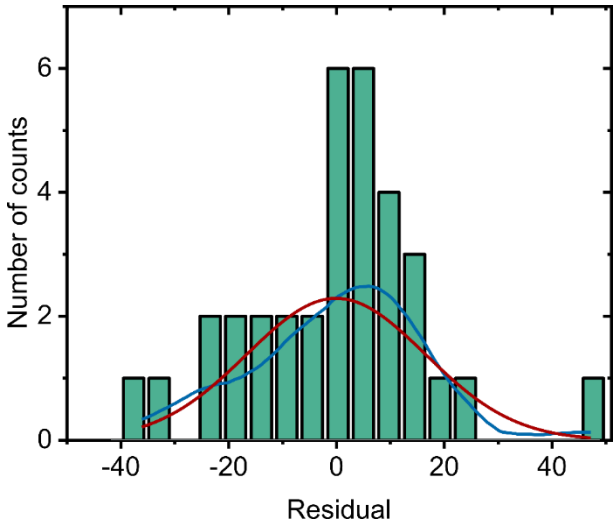


Figure S4. The histogram of the residuals of the model. Blue curve – Kernel density normalized to the number of counts, red curve – normal distribution.

According to the Breusch-Pagan test, we fail to reject the hypothesis of constant variance (homoskedasticity) at the significance level of 0.05. The same conclusion was received from White's test. At the same significance level, we also fail to reject the hypothesis of skewness and kurtosis of the distribution of residuals (**Figure S5**).

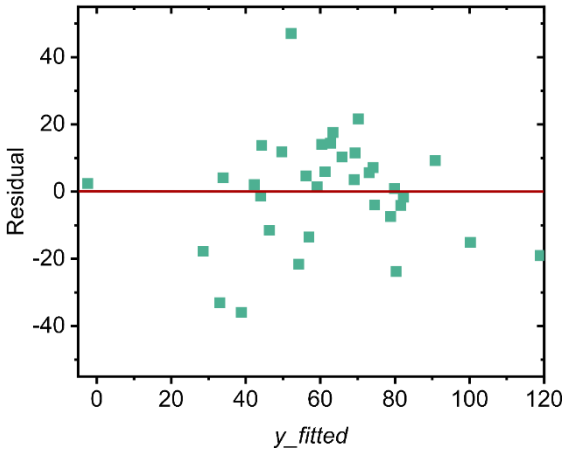
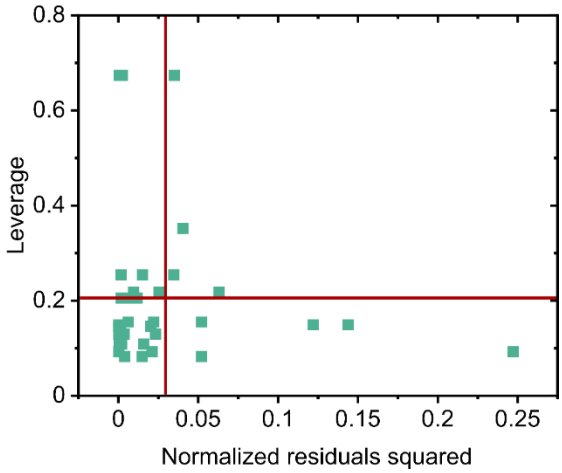


Figure S5. Residuals of the model for fitted values of y_i.

```
Breusch–Pagan/Cook–Weisberg test for heteroskedasticity
Assumption: Normal error terms
Variable: Fitted values of y

H0: Constant variance

    chi2(1) =   1.18
Prob > chi2 = 0.2767

White's test
H0: Homoskedasticity
Ha: Unrestricted heteroskedasticity

   chi2(21) =  21.22
Prob > chi2 = 0.4457

Cameron & Trivedi's decomposition of IM-test
```

Source	chi2	df	p
Heteroskedasticity	21.22	21	0.4457
Skewness	2.10	6	0.9103
Kurtosis	1.12	1	0.2889
Total	24.44	28	0.6580

No observations should be removed from the model because none of them was spotted in the leverage-normalized residual squared plot's upper-right corner (**Figure S6**). In other words, no observation strongly biases the estimated β-coefficients.



277

278 **Figure S6.** The leverages-normalized residual squared analysis.

279

280 ***Interpretation of the model***

281 The model did not pass the RESET, but passed the other tests according to the diagnostics.
282 However, it is essential to mention that the model was based only on 36 experiments (each
283 experiment consisted of 3 repetitions of 8 repetitions of the phage titration) and an additional
284 theoretical observation (base 100% activity of the certain bacteriophage after 24 h if any
285 polymer was added). Due to so few observations, the model may omit some important
286 information, even if the statistical analysis based on the available data is valid. This issue is also
287 visible in high standard errors of the parameters.

288 The final form of the model assumed independent variables and their impact on the
289 explained variable y_i (i.e., the activity of the bacteriophage after 24 h [%]):

290 $$y_i = \beta_0 + \beta_1 \cdot int\_t4\_zeta2 + \beta_2 \cdot int\_ms2\_zeta2 + \beta_3 \cdot int\_m13\_zeta2 + \beta_4 \cdot radius +$$

291 $$\beta_5 \cdot radius2 + \beta_6 \cdot coscotangle \quad (10)$$

292 60.75% of the variance of y is explained by the chosen explanatory variables. The
293 adjusted R^2 (correcting the positive bias related to the number of observations and number of
294 explanatory variables) shows that the model should explain 55.33% of the variance of y in the
295 population. The model's estimated parameters (coefficients) are given in **Table S3**.

Table S3. Estimated parameters (coefficients) of the model.

Estimated parameter	Value	Standard Error	Unit
β_0	119.0145	10.80451	-
β_1	-0.0009271	0.0025219	mV^{-2}
β_2	-0.0045919	0.0025219	mV^{-2}
β_3	-0.0087035	0.0025219	mV^{-2}
β_4	-3.368489	0.7750576	μm^{-1}
β_5	0.052013	0.0142138	μm^{-2}
β_6	-81.855	19.71572	-

- The constant value β_0 = 119.0145±10.80451 represents the basic level, theoretically 100% activity of the certain bacteriophage.
- For T4 bacteriophage, the activity drops 0.0009271±0.0025219 pp with the increase of 1 mV^2 of the squared zeta potential of the polymer.
- For MS2 bacteriophage, the activity drops 0.0045919±0.0025219 pp with the increase of 1 mV^2 of the squared zeta potential of the polymer.
- For M13 bacteriophage, the activity drops 0.0087035±0.0025219 pp with the increase of 1 mV^2 of the squared zeta potential of the polymer.
- The activity drops 3.368489±0.7750576 pp with the increase of 1 μm of the average radius of the plastic microparticle.
- The activity rises 0.052013±0.0142138 pp with the increase of 1 μm^2 of the average squared radius of the plastic microparticle.
- The activity drops 81.855±19.71572 pp for the $coscotangle$ = 1

The variable *radius* depends on two variables bet (the surface area of the polymer measured with BET method [$m^2 \cdot g^{-1}$]) and $density$ (the density of the polymer [$g^2 \cdot cm^{-3}$]) according to equation 6. The change of fitted value y_i depending on the *radius* is presented in **Figure S7**. Both very small and very large particles are not efficient in scavenging phages.

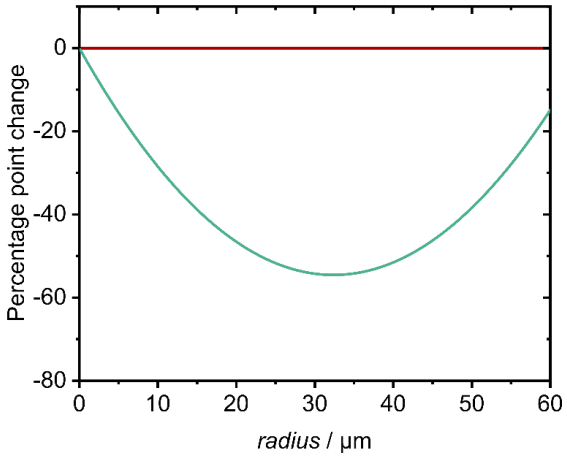


Figure S7. Change of the fitted y_i depending on the radius of the plastic microparticle.

2.4. Model 1B

Model 1B is modified **Model** 1A, i.e., without two variables: *int_t4_zeta2* and *int_ms2_zeta2*. They were removed because of too high a statistical probability that the β coefficients are equal to zero.

Source	SS	df	MS	Number of obs	=	34
				F(4, 29)	=	11.22
Model	15743.6002	4	3935.90004	Prob > F	=	0.0000
Residual	10173.5008	29	350.810372	R-squared	=	0.6075
				Adj R-squared	=	0.5533
Total	25917.101	33	785.366696	Root MSE	=	18.73

y	Coefficient	Std. err.	t	P>|t|	[95% conf.	interval]
int_m13_zeta2	-.0068718	.0021243	-3.23	0.003	-.0112164	-.0025272
radius	-3.646578	.7646678	-4.77	0.000	-5.210499	-2.082656
radius2	.0581181	.013755	4.23	0.000	.0299859	.0862504
cosconangle	-77.76235	20.00964	-3.89	0.001	-118.6866	-36.83805
_cons	114.2707	10.42509	10.96	0.000	92.94901	135.5924

F-statistic, R^2, adjusted R^2

As the F-statistic is equal to 11.22 and the p-value $\approx$ 0.0000, we can conclude that there is a statistically significant relationship between y and the chosen explanatory variables. 60.75% of the variance of y is explained by the chosen explanatory variables. The adjusted R^2 (correcting the positive bias related to the number of observations and number of explanatory variables) shows that the model should explain 55.33% of the variance of y in the population.

Ramsey RESET test

```
Ramsey RESET test for omitted variables
Omitted: Powers of fitted values of y

H0: Model has no omitted variables

F(3, 26) =   0.86
Prob > F = 0.4744
```

With the p-value of 0.4744, we fail to reject the hypothesis of the correct specification of the model (significance level of 0.05). On the significance level of 0.05, we fail to reject the

hypothesis that the estimated parameters of variables *int_m13_zeta2*, *cosconangle*, *radius,* and *radius2* are equal to zero.

Correlation Matrix

There is high collinearity between variables *radius* and *radius2*, because *radius2* = *radius*^{2}.

	int_m1~2	radius	radius2	coscon~e
int_m13_ze~2	1.0000			
radius	-0.0188	1.0000		
radius2	-0.0555	0.9577	1.0000	
cosconangle	-0.0447	-0.5215	-0.4895	1.0000

Multicollinearity

As expected *radius* and *radius2* present high VIF values, but they were introduced to the model on purpose. VIF values for other variables are much lower than 10.

Variable	VIF	1/VIF
radius	12.78	0.078218
radius2	12.31	0.081247
cosconangle	1.38	0.724308
int_m13_ze~2	1.02	0.979193
Mean VIF	6.87	

Residuals analysis and homoskedasticity

According to the Shapiro-Wilk test, we fail to reject the 0-hypothesis of the normal distribution of residuals in the model at the significance level of 0.05 (p-value = 0.14477). The distribution is presented in **Figure S8**. The Kernel density plot (normalized to the number of counts) almost matches the Gaussian curve. There was no strong skewness, nor was kurtosis noticed (too few observations may cause it).

Shapiro-Wilk W test for normal data

Variable	Obs	W	V	z	Prob>z
e	34	0.95239	1.662	1.059	0.14477

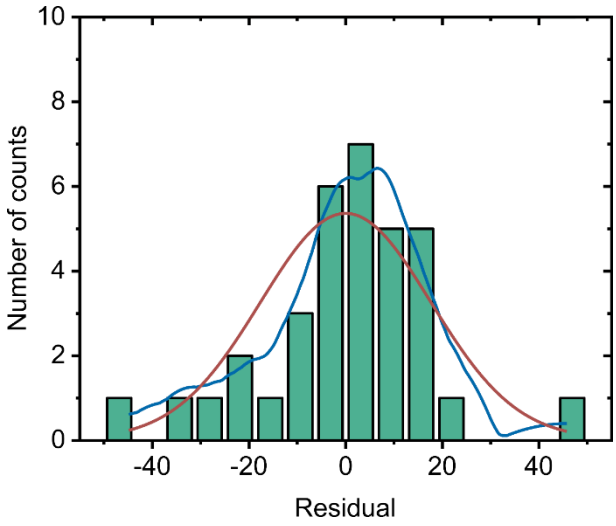


Figure S8. The histogram of the residuals of the model. Blue curve – Kernel density normalized to the number of counts, red curve – normal distribution.

According to the Breusch-Pagan test, we fail to reject the hypothesis of constant variance (homoskedasticity) at the significance level of 0.05. The same conclusion was received from White's test. At the same significance level, we also fail to reject the hypothesis of skewness and kurtosis of the distribution of residuals (**Figure S9**).

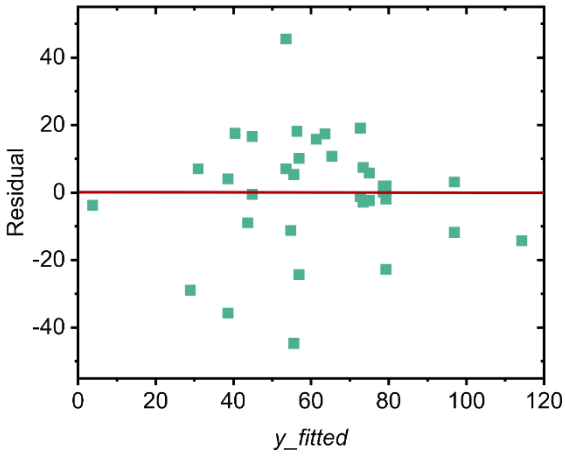


Figure S9. Residuals of the model for fitted values of y_i.

```
Breusch-Pagan/Cook-Weisberg test for heteroskedasticity
Assumption: Normal error terms
Variable: Fitted values of y

H0: Constant variance

    chi2(1) =   2.01
Prob > chi2 = 0.1568
```

```
White's test
H0: Homoskedasticity
Ha: Unrestricted heteroskedasticity

   chi2(13) =  11.54
Prob > chi2 = 0.5658

Cameron & Trivedi's decomposition of IM-test
```

Source	chi2	df	p
Heteroskedasticity	11.54	13	0.5658
Skewness	1.89	4	0.7561
Kurtosis	2.47	1	0.1159
Total	15.90	18	0.5996

No observations should be removed from the model because none of them was spotted in the leverage-normalized residual squared plot's upper-right corner (**Figure S10**). In other words, there is no observation that strongly bios the estimated β-coefficients.

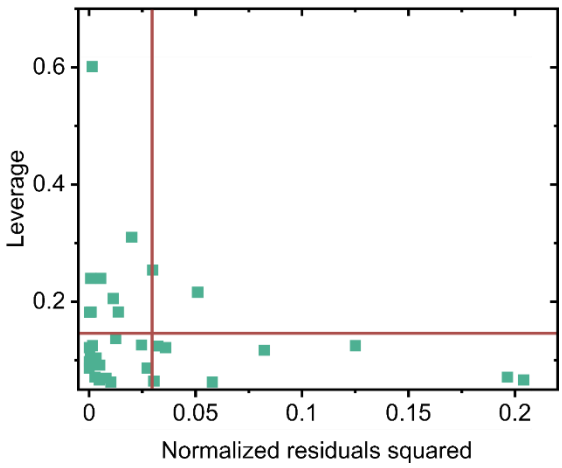


Figure S10. The leverages-normalized residual squared analysis.

Interpretation of the model

According to the diagnostics, the model seems to be valid for drawing nonbiased conclusions on the described phenomenon. However, it is important to mention that the model was based only on 36 experiments (each experiment consisted of 3 repetitions of 8 repetitions of the phage titration) and the additional theoretical observation (base 100% activity of the specific bacteriophage after 24 h if any polymer was added). Due to so few observations, the model may omit some important information, even if the statistical analysis based on the available data is valid. This issue is also visible in relatively high standard errors of the estimated parameters.

The final form of the model assumes independent variables and their impact on the explained variable y_i (the activity of the bacteriophage after 24 h [%]):

$$y_i = \beta_0 + \beta_1 \cdot int\_m13\_zetasqr + \beta_2 \cdot radius + \beta_3 \cdot radius2 + \beta_4 \cdot coscotangle \quad (11)$$

60.75% of the variance of y is explained by the chosen explanatory variables. The adjusted R^2 (correcting the positive bias related to the number of observations and number of explanatory variables) shows that the model should explain 55.33% of the variance of y in the population. The model's estimated parameters (coefficients) are given in **Table S4**.

Table S4. Estimated parameters (coefficients) of the model.

Estimated parameter	Value	Standard Error	Unit
β_0	114.2707	10.42509	-
β_1	-0.0068718	0.0021243	mV^{-2}
β_2	-3.646578	0.7646678	μm^{-1}
β_3	0.0581181	0.013755	μm^{-2}
β_4	-77.76235	20.00964	-

- The constant value β_0 = 114.2707±10.42509 represents the basic level of 100% activity of the certain bacteriophage.
- For M13 bacteriophage, the activity drops 0.0068718±0.0021243 pp with the increase of 1 mV^2 of the squared zeta potential of the polymer.
- The activity drops 3.646578±0.7646678 pp with the increase of 1 μm of the average radius of the plastic microparticle.
- The activity rises 0.0581181±0.013755 pp with the increase of 1 μm^2 of the squared average radius of the plastic microparticle.

The change of fitted value y_i depending on the *radius* is presented in **Figure S11** (*cf.* **Figure S7**).

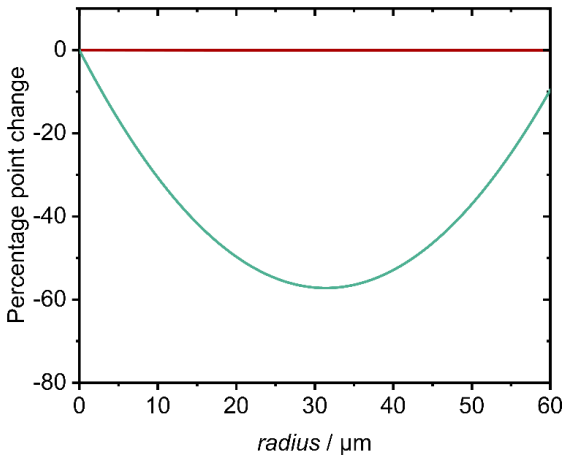


Figure S11. Change of the fitted y_i depending on the radius of the plastic microparticles.

2.5. Model 2

Model 2 was based on the coarse estimation, where phages were replaced by interactions between the phage type and zeta potential squared.

Source	SS	df	MS	Number of obs	=	37
				F(14, 22)	=	8.01
Model	21932.2554	14	1566.58967	Prob > F	=	0.0000
Residual	4301.32214	22	195.514643	R-squared	=	0.8360
				Adj R-squared	=	0.7317
Total	26233.5775	36	728.710487	Root MSE	=	13.983

y	Coefficient	Std. err.	t	P>|t|	[95% conf.	interval]
int_t4_zeta2	-.0062459	.0024041	-2.60	0.016	-.0112317	-.0012602
int_ms2_zeta2	-.0100222	.0024041	-4.17	0.000	-.015008	-.0050365
int_m13_zeta2	-.0140335	.0024041	-5.84	0.000	-.0190192	-.0090477
abs	-31.58638	13.20681	-2.39	0.026	-58.97562	-4.197135
hips	-8.059362	13.34243	-0.60	0.552	-35.72987	19.61115
pa6	-8.008128	13.98612	-0.57	0.573	-37.01356	20.9973
pc	4.15582	12.62334	0.33	0.745	-22.02339	30.33502
pe	3.165382	12.78384	0.25	0.807	-23.34668	29.67745
pet	-70.60276	14.02794	-5.03	0.000	-99.69493	-41.5106
pmma	-1.273046	13.20681	-0.10	0.924	-28.66229	26.1162
pp	-4.605286	12.14838	-0.38	0.708	-29.79948	20.58891
ps	-35.79936	13.34243	-2.68	0.014	-63.46987	-8.128851
ptfe	6.397717	12.56614	0.51	0.616	-19.66287	32.4583
pur	-19.40862	12.63479	-1.54	0.139	-45.61157	6.794334
pvc	0	(omitted)				
_cons	100	13.98266	7.15	0.000	71.00175	128.9983

Next, the unnecessary variables were removed (i.e., those whose β coefficients might be equal to 0 due to statistical analysis). This action aimed to determine for which polymers the DLVO theory is not sufficient.

Source	SS	df	MS	Number of obs	=	37
				F(7, 29)	=	17.96
Model	21317.3771	7	3045.33959	Prob > F	=	0.0000
Residual	4916.20043	29	169.524153	R-squared	=	0.8126
				Adj R-squared	=	0.7674
Total	26233.5775	36	728.710487	Root MSE	=	13.02

y	Coefficient	Std. err.	t	P>|t|	[95% conf.	interval]
int_t4_zeta2	-.0057495	.0017333	-3.32	0.002	-.0092945	-.0022044
int_ms2_zeta2	-.0095258	.0017333	-5.50	0.000	-.0130708	-.0059807
int_m13_zeta2	-.013537	.0017333	-7.81	0.000	-.017082	-.0099919
abs	-30.21608	8.028997	-3.76	0.001	-46.63722	-13.79493
pet	-68.92525	8.191872	-8.41	0.000	-85.67951	-52.17099
ps	-34.37615	8.050589	-4.27	0.000	-50.84145	-17.91085
pur	-18.27415	7.966349	-2.29	0.029	-34.56716	-1.981135
_cons	97.62429	4.714613	20.71	0.000	87.98182	107.2668

F-statistic, R^2, adjusted R^2

As the F-statistic is equal to 17.96 and the p-value ≈ 0.0000, we can conclude a statistically significant relationship between y and the chosen explanatory variables. 81.26% of the variance of y is explained by the chosen explanatory variables. The adjusted R^2 (correcting the positive bias related to the number of observations and number of explanatory variables) shows that the model should explain 76.74% of the variance of y in the population.

Ramsey RESET test

```
Ramsey RESET test for omitted variables
Omitted: Powers of fitted values of y

H0: Model has no omitted variables

F(3, 26) =   2.50
Prob > F = 0.0820
```

With a p-value of 0.0820, we fail to reject the hypothesis of the correct specification of the model (significance level of 0.05). However, we did not fail to reject the hypothesis on the significance level of 0.10. On the significance level of 0.05, we failed to reject the hypothesis that estimated parameters are equal to zero.

Correlation Matrix

There is no high collinearity between any variables.

	int_t4~2	~2_zeta2	int_m1~2	abs	pet	ps	pur
int_t4_zeta2	1.0000						
int_ms2_ze~2	-0.3139	1.0000					
int_m13_ze~2	-0.3139	-0.3139	1.0000				
abs	-0.0330	-0.0330	-0.0330	1.0000			
pet	-0.0738	-0.0738	-0.0738	-0.0882	1.0000		
ps	-0.0400	-0.0400	-0.0400	-0.0882	-0.0882	1.0000	
pur	-0.0017	-0.0017	-0.0017	-0.0882	-0.0882	-0.0882	1.0000

Multicollinearity

VIF values are much lower than 10.

Variable	VIF	1/VIF
int_m13_ze~2	1.48	0.675259
int_ms2_ze~2	1.48	0.675259
int_t4_zeta2	1.48	0.675259
pet	1.09	0.916362
ps	1.05	0.948807
abs	1.05	0.953917
pur	1.03	0.968979
Mean VIF	1.24	

431

432 **Residuals analysis and homoskedasticity**

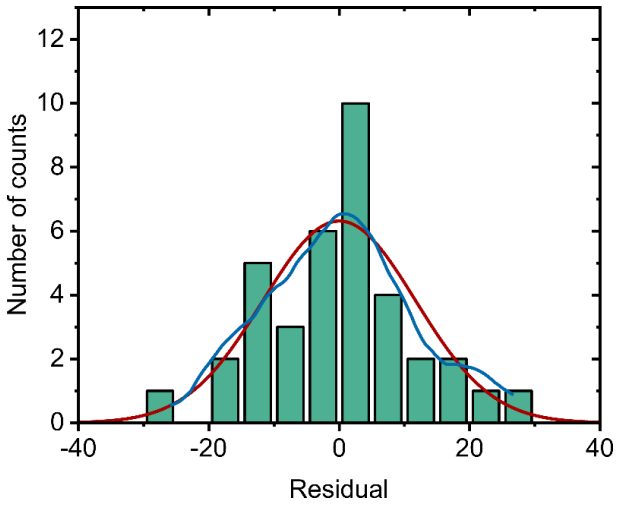
433 According to the Shapiro-Wilk test, we fail to reject the 0-hypothesis of the normal distribution
434 of residuals in the model at the significance level of 0.05 (p-value = 0.83799). The distribution
435 is presented in **Figure S12**. The Kernel density plot (here normalized to a number of counts)
436 almost matches the Gaussian curve. No strong skewness or kurtosis were noticed.

Shapiro-Wilk W test for normal data

Variable	Obs	W	V	z	Prob>z
e	37	0.98323	0.624	-0.986	0.83799

437

438



439

440 **Figure S12.** The histogram of the residuals of the model. Blue curve – Kernel density
441 normalized to the number of counts, red curve – normal distribution.

442

According to the Breusch-Pagan test, we fail to reject the hypothesis of constant variance (homoskedasticity) at the significance level of 0.05. The same conclusion we received from White's test. At the same significance level, we also fail to reject the hypothesis of skewness and kurtosis of the distribution of residuals (**Figure S13**).

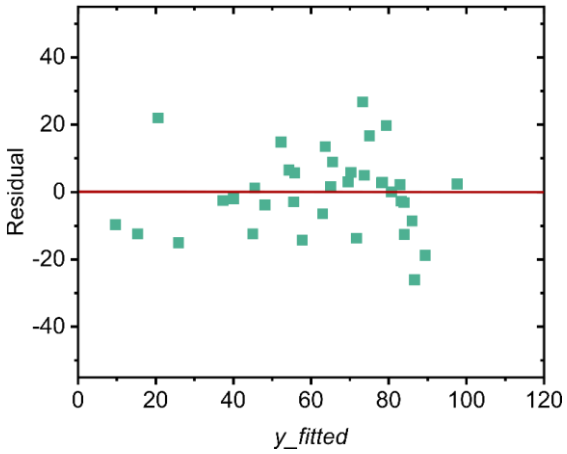


Figure S13. Residuals of the model for fitted values of y_i.

```
Breusch-Pagan/Cook-Weisberg test for heteroskedasticity
Assumption: Normal error terms
Variable: Fitted values of y

H0: Constant variance

    chi2(1) =   0.14
Prob > chi2 = 0.7054
```

```
White's test
H0: Homoskedasticity
Ha: Unrestricted heteroskedasticity

   chi2(18) =  10.01
Prob > chi2 = 0.9315

Cameron & Trivedi's decomposition of IM-test
```

Source	chi2	df	p
Heteroskedasticity	10.01	18	0.9315
Skewness	6.25	7	0.5107
Kurtosis	0.07	1	0.7855
Total	16.34	26	0.9277

No observations should be removed from the model because none of them was spotted in the upper-right corner of the leverage-normalized residuals squared plot (**Figure S14**). In other words, there is no observation that strongly bios the estimated β-coefficients.

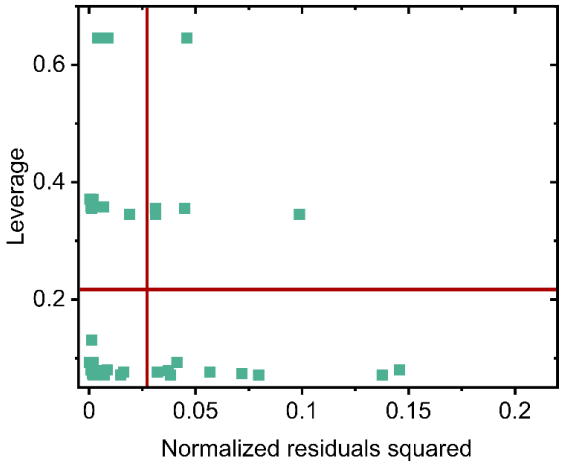


Figure S14. The leverages-normalized residual squared analysis.

To prove it, the observation visible in the middle of the graph was removed. The model B – building was repeated, leading to the same form of the model.

Source	SS	df	MS		
				Number of obs	= 36
				F(7, 28)	= 17.02
Model	20918.7871	7	2988.39815	Prob > F	= 0.0000
Residual	4916.19373	28	175.578348	R-squared	= 0.8097
				Adj R-squared	= 0.7621
Total	25834.9808	35	738.142309	Root MSE	= 13.251

y	Coefficient	Std. err.	t	P>|t|	[95% conf.	interval]
int_t4_zeta2	-.0057511	.0017851	-3.22	0.003	-.0094078	-.0020945
int_ms2_zeta2	-.0095251	.0017668	-5.39	0.000	-.0131443	-.0059059
int_m13_zeta2	-.0135363	.0017668	-7.66	0.000	-.0171556	-.0099171
abs	-30.21276	8.188771	-3.69	0.001	-46.98669	-13.43882
pet	-68.92203	8.353212	-8.25	0.000	-86.03281	-51.81125
ps	-34.37285	8.210527	-4.19	0.000	-51.19135	-17.55435
pur	-18.27076	8.125935	-2.25	0.033	-34.91598	-1.625533
_cons	97.62128	4.82278	20.24	0.000	87.74226	107.5003

This operation resulted in a minuscule decrease in the standard errors of the coefficients. It also decreased the R^2.

Interpretation of the model:

According to the diagnostics, the model seems valid for drawing nonbiased conclusions on the described phenomenon. However, it is essential to mention that the model was based only on 36 experiments (each experiment consisted of 3 repetitions of 8 repetitions of the phage titration) and the additional theoretical observation (base 100% activity of the specific bacteriophage after 24 h if any polymer was added). Due to so few observations, the model may omit some important information, even if the statistical analysis based on the available data is valid. This issue is also visible in relatively high standard errors of the estimated parameters.

The final form of the model assumes independent variables and their impact on the explained variable y_i (the activity of the bacteriophage after 24 h [%]):

$$y_i = \beta_0 + \beta_1 \cdot int\_t4\_zeta2 + \beta_2 \cdot int\_ms2\_zeta2 + \beta_3 \cdot int\_m13\_zeta2 + \beta_4 \cdot abs + \beta_5 \cdot pet + \beta_6 \cdot ps + \beta_7 \cdot pur \quad (12)$$

81.26% of the variance of y is explained by the chosen explanatory variables. The adjusted R^2 (correcting the positive bias related to the number of observations and number of explanatory variables) shows that the model should explain 76.74% of the variance of y in the population. The model's estimated parameters (coefficients) are given in **Table S4**.

Table S4. Estimated parameters (coefficients) of the model.

Estimated parameter	Value	Standard Error	Unit
β_0	97.62429	4.714613	-
β_1	-0.0057495	0.0017333	mV^{-2}
β_2	-0.0095258	0.0017333	mV^{-2}
β_3	-0.0135370	0.0017333	mV^{-2}
β_4	-30.21608	8.028997	-
β_5	-68.92525	8.191872	-
β_6	-34.37615	8.050589	-
β_7	-18.27415	7.966349	-

- The constant value β_0 = 97.62429±4.714613 represents the basic level of 100% activity of the specific bacteriophage.
- For T4 bacteriophage, the activity drops 0.0057495±0.0017333 pp with the increase of 1 mV^2 of the squared zeta potential of the polymer.
- For MS2 bacteriophage, the activity drops 0.0095258±0.0017333 pp with the increase of 1 mV^2 of the squared zeta potential of the polymer.
- For M13 bacteriophage, the activity drops 0.0135370±0.0017333 pp with the increase of 1 mV^2 of the squared zeta potential of the polymer.
- The activity drops 30.21608±8.028997 pp if in the experiment ABS was used.
- The activity drops 68.92525±8.191872 pp if in the experiment PET was used.
- The activity drops 34.37615±8.050589 pp if in the experiment PS was used.
- The activity drops 18.27415±7.966349 pp if in the experiment PUR was used.

This model shows that the activity of the bacteriophage suspension can be explained by the interaction between the type of the phage and the zeta potential of the polymer. It is sufficient for most polymers except for ABS, PET, PS, and PUR, which, if present, decreases the activity of the bacteriophage suspension.

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