

Supplementary Information for:

**Enhanced antimicrobial effect of berberine in nanogel carriers with
cationic surface functionality**

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(J. Mater. Chem. B)

Contents

Swelling behaviour of Carbopol Aqua SF1 nanogel.....	3
Particle size and electrophoretic mobility of the nanogel at various pH	6
Effect of Carbopol concentration on berberine encapsulation.....	9
Effect of the berberine concentration on the Carbopol stability.....	10
Determination of the berberine-loaded Carbopol nanogel particle size.....	11
Functionalization of Carbopol and berberine-loaded Carbopol	13
Effect of the PDAC concentration on the Carbopol nanogel coating.....	17
Cationic functionalization of antimicrobial-loaded Carbopol nanogel.....	18
UV-Visible spectrophotometry of berberine-loaded Carbopol.....	20
FTIR spectrometry of berberine loaded Carbopol.....	21
Thermogravimetric analysis of berberine loaded Carbopol.....	23
Elemental analysis of berberine-loaded Carbopol Aqua SF1 nanogel.....	24
Berberine encapsulation efficiency and loading content in Carbopol SF1 nanogel.....	25
Berberine release from berberine-loaded Carbopol nanogel suspensions.....	26
Effect of the non-loaded Carbopol nanogel on the cell viability.....	28
TEM of microalgae after being incubated with non-loaded Carbopol nanogel.....	29
References.....	30

Swelling behaviour of Carbopol Aqua SF1 nanogel

Carbopol Aqua SF1 nanogel are known to change their particle size by swelling, depending on the ionic strength and pH of the solution. To quantify this phenomenon aliquots of Carbopol Aqua SF1 were prepared and adjusted in a range of pH from 5 to 12 using small amounts of 0.25 M sodium hydroxide. The average particle diameters of the collapsed and swollen Carbopol Aqua SF1 nanogels were determined using Malvern Mastersizer instrument at the refractive indexes of 1.453 and 1.336, respectively. The corrected particle size and refractive index of the swollen nanogel particles were calculated by combining the data from the refractometer and the Mastersizer measurements as follows. At low pH, where the nanogel was collapsed, assuming that at this pH the micro gel is pure polyacrylic acid (PAA) then:

$$v_o = \frac{\pi}{6} d_o^3. \quad (1)$$

Here v_o and d_o are the volume and the diameter of the collapsed Carbopol Aqua SF1 nanogel particles (at pH 5 for example). At high pH the nanogel is swollen with water and consequently in one particle of nanogel there is both PAA and water. The volume of this particles is then:

$$v = \frac{\pi}{6} d^3. \quad (2)$$

Here v is the volume of swollen Carbopol Aqua SF1 particles at pH 8 for example; d is the diameter of swollen carbopol particles (at pH 8 for example). Assuming ϕ_a is the volume fraction of PAA in the swollen micro-gel particle and ϕ_w is the volume fraction of water:

$$\phi_a = \frac{v_o}{v} = \left(\frac{d_o}{d}\right)^3 \quad \text{and} \quad \phi_w = 1 - \phi_a = 1 - \left(\frac{d_o}{d}\right)^3 \quad (3)$$

Furthermore if m_a is the mass of PAA in the swollen micro-gel particle, m_w is the mass of water in the swollen micro-gel particle; ρ_w and ρ_a are the mass densities of water and PAA, respectively.

$$m_a = v\phi_a\rho_a \dots\dots\dots (4)$$

$$m_w = v\phi_w\rho_w \dots\dots\dots (5)$$

Finally for the weight percentage of PAA W_a we can write:

$$w_a = \frac{m_a}{m_a + m_w} \times 100 \quad \dots\dots\dots(6)$$

Then, by substituting Eqs (4) and (5) into Eq. (6), the weight percentage of PAA in the swollen micro-gel particle can be calculated to be

$$w_a = \frac{100 \nu \phi_a \rho_a}{\nu \phi_a \rho_a + \nu \phi_w \rho_w} = \frac{100 \phi_a \rho_a}{\phi_a \rho_a + \phi_w \rho_w} \quad (7)$$

After that we substituted Eq. (3) (3) in Eq. (7) to obtain:

$$w_a = \frac{100 d_0^3 \rho_a}{d_0^3 (\rho_a - \rho_w) + \rho_w d^3} \quad \dots(8)$$

The particle size of the collapsed Carbopol Aqua SF1 nanogel particles is $d_0 = 100$ nm which can be obtained from equation (8), and the mass densities of PAA and water can be taken from the literature^{1,2} $\rho_w = 1$ g cm⁻³ and $\rho_a = 1.15$ g cm⁻³. The refractive index of the Carbopol Aqua SF1 suspension was measured using Abbey refractometer with 589 nm sodium line at room temperature. The experiment included measurement of the suspension refractive index at different concentrations of Carbopol Aqua SF1. The linear regression equation for the calibration is:

$$n_s = n_w + \alpha w_a \quad (9)$$

Here $n_w = 1.3325$ is the refractive index of water at room temperature and $\alpha = 0.0012$ is the slope of the experimental data plot of the refractive index of the carbopol Aqua SF1 solution, n_s , versus the PAA weight fraction in the suspension, w_a .

Since the nanogel particles swell upon changing the pH from 5 to 8 their particle refractive index also changes. Since the refractive index of the swollen nanogel particle is unknown, this means that the particles diameter of the swollen nanogel are unrealistic if estimated at fixed refractive index of PAA above pH 8. Therefore, the average particle diameter of a specific concentration of Carbopol Aqua SF1 nanogel (0.3 wt%) was determined as a function of refractive index. The linear regression equation for the calibration was:

$$d = d_1 + \beta n_s, \quad (10)$$

where the model parameters $d_1 = 0.6415$ and $\beta = -0.2323$ are determined by fitting the diameter of the particles versus the refractive index of the suspension.

To work out the real size of the swollen nanogel particles at pH 8, the particles refractive index (n_p) can be approximated as the dispersion refractive index (n_s) so that the weight fraction of PAA can be calculated.

$$n_p = n_s.$$

Hence, equation (9) become,

$$n_p = n_w + \alpha w_a = n_w + \alpha \frac{100 \phi_a \rho_a}{\phi_a(\rho_a - \rho_w) + \rho_w} \dots \dots \dots (11)$$

By substituting equation (11) in equation (10), we obtained

$$d = d_1 + \beta \left(n_w + \alpha \frac{100 \phi_a \rho_a}{\phi_a(\rho_a - \rho_w) + \rho_w} \right) \dots \dots \dots (12)$$

$$\frac{d}{d_0} = \frac{d_1 + \beta \left(n_w + \alpha \frac{100 \phi_a \rho_a}{\phi_a(\rho_a - \rho_w) + \rho_w} \right)}{d_0} \dots \dots \dots (13)$$

From equation (3), we obtain $\phi_a = \left(\frac{d_0}{d} \right)^3$, so $\frac{d}{d_0} = \frac{1}{\phi_a^{1/3}}$

By combining the previous equation with Eq. (13),

$$\frac{1}{\phi_a^{1/3}} = \frac{d_1}{d_0} + \frac{\beta}{d_0} \left(n_w + \alpha \frac{100 \phi_a \rho_a}{\phi_a(\rho_a - \rho_w) + \rho_w} \right) \dots \dots \dots (14)$$

Hence we finally obtain:

$$\phi_a = 0.02592$$

$$d = \frac{d_o}{\phi_a^{1/3}} = \frac{0.1}{\sqrt[3]{0.02592}} = 0.334 \mu m$$

The weight fraction of PAA inside the swollen nanogel particles (w_a) is therefore 2.6 % while the rest is water. In addition of that, the refractive index of swollen nanogel particles (n_p) is 1.336. In the above calculations, it was used two different refractive indexes due to the fact that as the Carbopol Aqua SF1 nanogel swells, the refractive index changes as the pH increases from 5 to 8. It is therefore important to notice that at low pH (below approximately 7), the nanogel particles were collapsed and their refractive index used was 1.453. When the pH increases Carbopol Aqua SF1 dispersion starts to form a gel, the solution became clear and the refractive index changes. In this case the refractive index used in Mastersizer was 1.336. 0.3 wt% w/w aqueous solution of Carbopol Aqua SF1 was prepared by weighing 1.5 g in 500 ml volumetric flask which completed up to the mark with purified water. 40 ml of the stock solution was added into each sample tube of eight 50 ml centrifuge tubes. The 40 ml of each centrifuge tube was adjusted to have a range of solution from pH 5 to pH 12 (0.1 M NaOH and 0.1 M HCl were used to adjust the medium). Then the particle size distribution was measured with Mastersizer 2000 MS (1000 rpm, Malvern Instruments, UK) for each pH solution at a fixed refractive index 1.453. The machine was washed three times using Milli Q water after each measurement. Then the samples were diluted to the right laser obscuration levels using the Milli-Q water.

Particle size and electrophoretic mobility studies of Carbopol Aqua SF1 nanogel particles at various pH

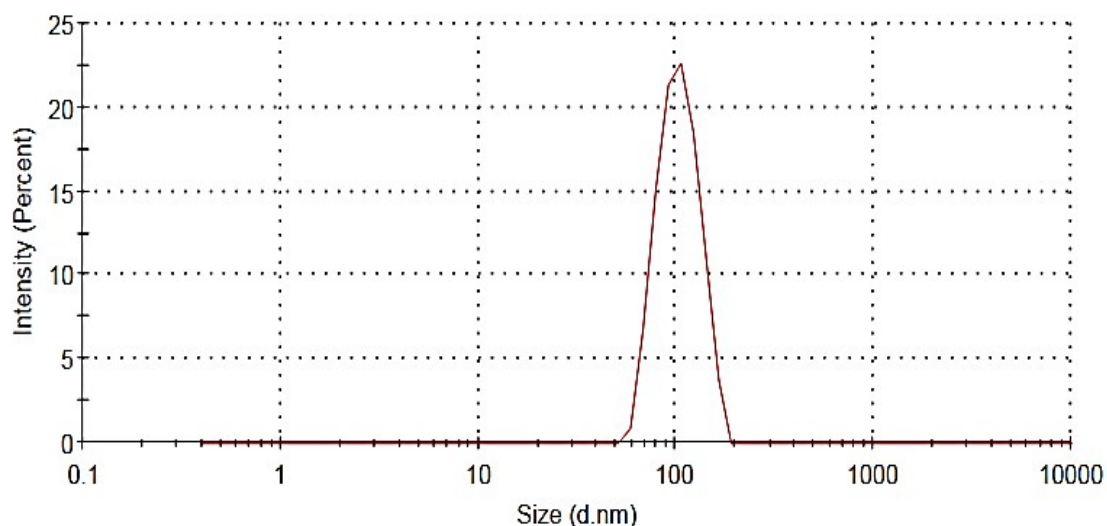


Figure S1. The average particle diameter of 0.05 wt% Carbopol Aqua SF1 prepared from 30 wt% stock solution (as supplied by the manufacturer) at pH 5.

Carbopol Aqua SF1 is a cloudy white aqueous suspension of a carboxyl groups functionalized cross-linked polyacrylic acid polymer³ with dendrimers like structure.⁴ Aliquots of the suspended Carbopol Aqua SF1 solution were prepared from the stock solution and used to measure the particle size and zeta potential using the Malvern Zetasizer Nano ZS instrument at a refractive index of the collapsed nanogel particles of 1.45 at room temperature. Figure S1 shows that the average particle diameter of Carbopol Aqua SF1 was found to be approximately 100 nm while the zeta potential is -44 mV which means that the Carbopol Aqua SF1 nanogel particles have high stability³ when suspended in MilliQ water at pH 5.5 as shown in Figure S2.

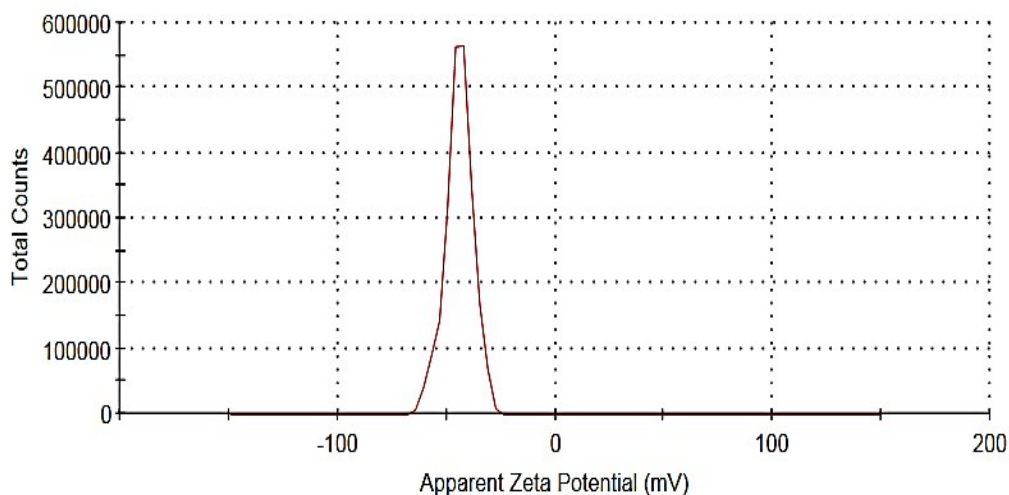


Figure S2. The zeta potential of 0.05 wt% Carbopol Aqua SF1 (collapsed nanogel particles) at pH 5.5 prepared from 30 wt% stock solution (as supplied by the manufacturer).

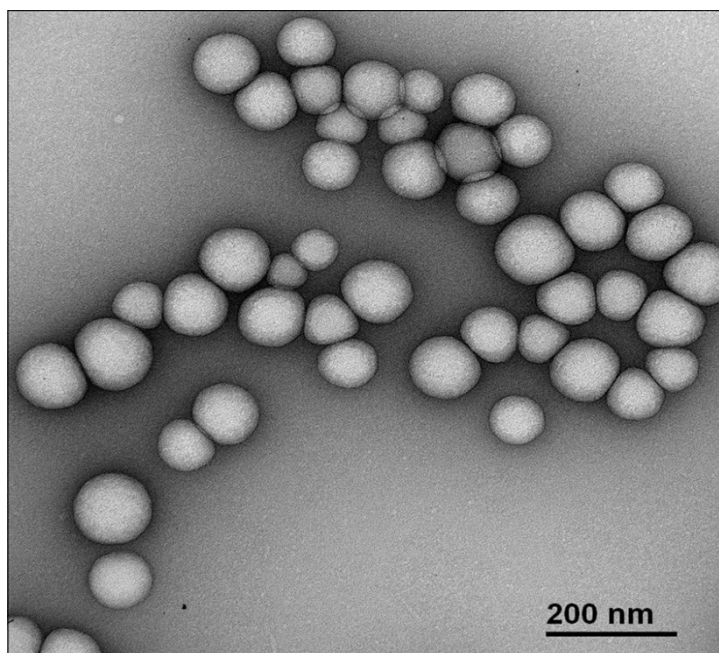


Figure S3. TEM image of Carbopol Aqua SF1 particles after drying of the suspension.

Figure S3 shows a typical TEM image of the dried up suspension of collapsed Carbopol Aqua SF1 nanogel particles. It can be seen that it contains spherical particles of about 100 ± 20 nm. This is in agreement with the average particle diameter measured by the Zetasizer instrument.

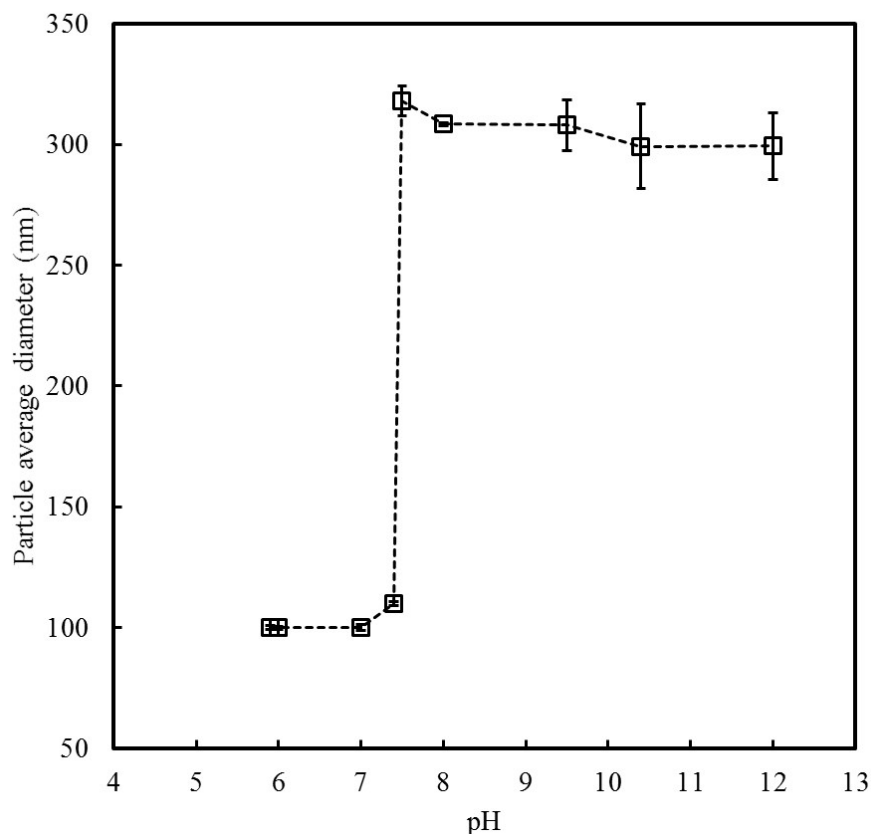


Figure S4. The average particle diameter of Carbopol Aqua SF1 as a function of pH. The size is calculated using variable refractive index of the particles in the Malvern Mastersizer, one is at the collapsed carbopol nanogel which is 1.453 while the refractive index is 1.336 in case of fully swollen Carbopol nanogel at pH 8. Aliquot of 0.25 M sodium hydroxide was used to adjust the pH. Each reading was repeated six times.

It also seems that in the collapsed form, the Carbopol Aqua SF1 particle contains protonated carboxylic groups and remains collapsed up to pH 7 without any noticeable change in particle size. Whereas, as the pH increased above 7, particles swelled leading to the increase in the particle size. The swelling behavior occurred because of the deprotonation of Carbopol Aqua SF1 particles which led to electrostatic repulsion between the dissociated carboxylic groups on the PAA chains. This leads to the partial swelling in the nanogel particles and the absorption of enormous amounts of aqueous phase from the surrounding solution. At high nanogel concentration at pH higher than 7, this leads to the formation of a highly viscous colourless gel due to the solution being effectively filled in with swollen nanogel particles. This can clearly be seen in in Figure S4 where from pH 5 up to pH 7, the average particle diameter is approximately constant at 100 nm. However, as the pH increases, the carbopol starts converting to the colourless nanogel suspension and the average particle size very steeply increases to

more than 300 nm. This behaviour of the Carbopol Aqua SF1 nanogel is comparable to the other pH responsive hydrogels reported in the literature.⁵

The swelling/deswelling feature of the Carbopol nanogel can be beneficially exploited for the purposes of development of drug delivery systems. This nanogel was therefore used to encapsulate antimicrobial agents like berberine chloride with the aim of sustained delivery and slow release of these agent to microbial cells.

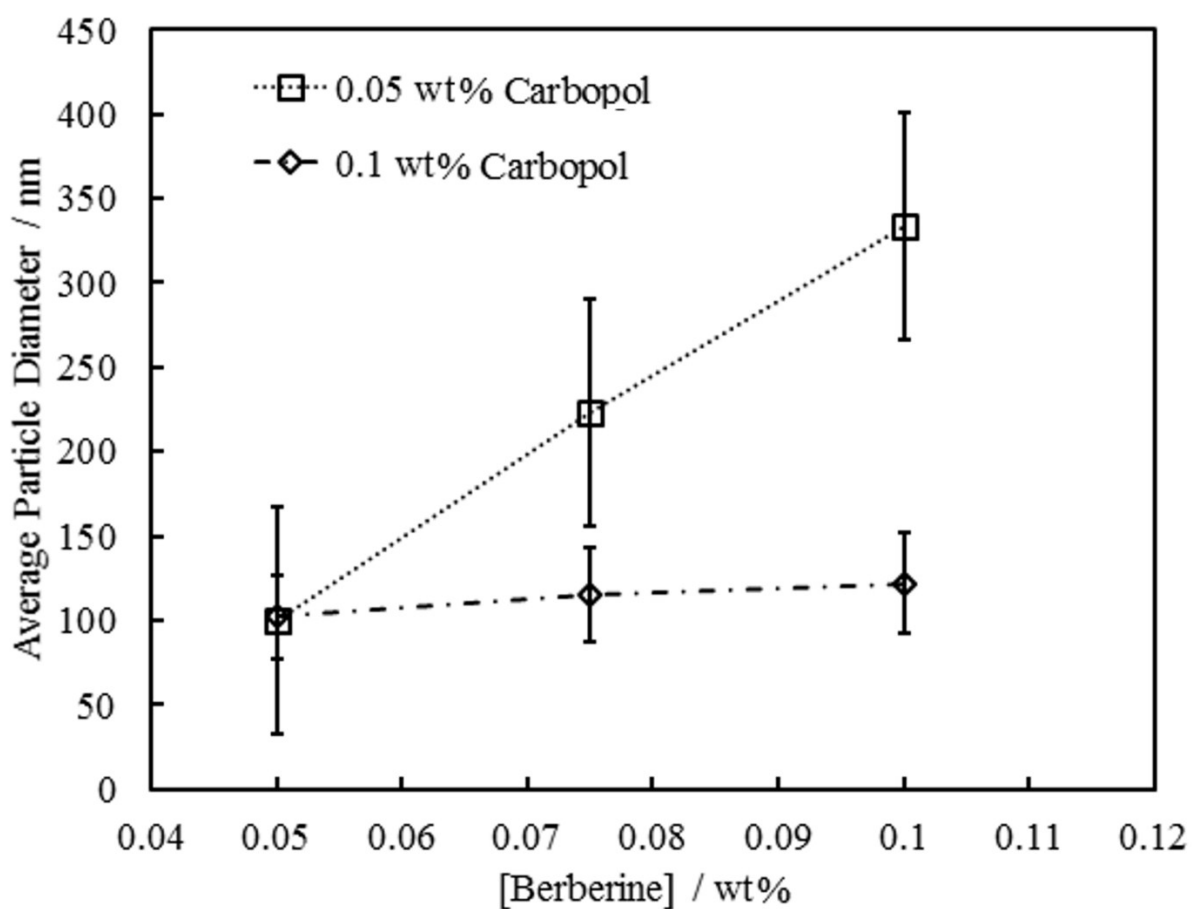


Figure S5. Average particle diameter of Carbopol nanogel particles following encapsulation of berberine at two different concentrations of Carbopol Aqua SF1.

Effect of Carbopol concentration on berberine encapsulation

Solutions of different concentrations of berberine were mixed with a dispersion of fixed concentration of Carbopol Aqua SF1, then stirred and its pH was decreased to 5.5, centrifuged and re-dispersed in Milli-Q water. The pH was then increased to pH 8, stirred overnight and then decreased to pH 5.5 with acetate buffer solution. The average particle diameter was measured for the berberine loaded Carbopol Aqua SF1 suspension at a refractive index for Carbopol Aqua SF1 of 1.456 at room temperature. Figure S5 shows the effect of the Carbopol Aqua SF1 concentration on the nanogel particle size of the berberine-loaded Carbopol Aqua SF1 particles. It can be seen that at 0.10 wt% Carbopol Aqua SF1 the particle size remains low whereas at 0.05 wt% Carbopol Aqua SF1 the particle size increases due to aggregation at high concentrations of berberine.

Effect of the berberine concentration on the Carbopol stability

The effect of the berberine concentration on the colloidal stability of Carbopol Aqua SF1 upon encapsulation of berberine into the Carbopol Aqua SF1 was then studied. Figure S6 shows the average nanogel particle diameter (purple Line) and their zeta potential (black line) of Carbopol Aqua SF1 as a function of berberine concentration. Please note that the term zeta-potential of a nanogel particle is not precisely defined due to the “soft” character of the nanogel particle when swollen with the surrounding media. Thus, at pH above 7 the position of the plane of shear is not well defined as the particles can deform as they move electrophoretically and values of the zeta-potential are for indicative purposes only. However, at pH below 7 the nanogel particles (including the ones loaded with Berberine) can be treated as solid particles with well-defined zeta potential and plane of shear. As the berberine concentration increases it can be seen that the average particle diameter also increases. Up to 0.15 wt% of berberine, the average particle diameter of Carbopol Aqua SF1 gradually increases but from 0.15 wt% to 0.2 wt%, no change in the particle size was noticed whereby the particle size is around 133 nm. We were not able to increase further the berberine solution concentration due to reaching its solubility limit. It can also be seen in Figure S6 that zeta potential of Carbopol Aqua SF1 decreases slightly as the berberine concentration rises. At very low concentration of berberine, the zeta potential of Carbopol Aqua SF1 is negative, -44 mV, which means high electrostatic stability of the colloid while it also stays stable at high concentration of berberine where the zeta potential of the nanogel at 0.2 wt % is -40 ± 5 mV.

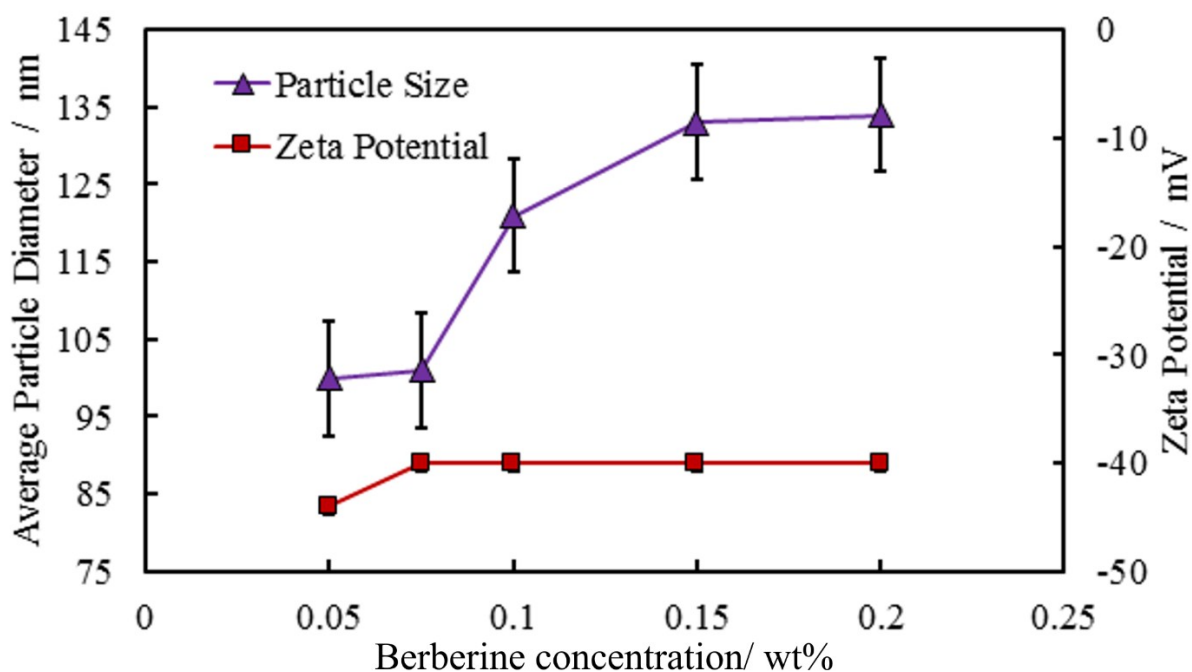


Figure S6. The average particle diameter and the zeta potential of Carbopol aqua SF1 as a function of berberine concentrations at pH 5.5 using 0.1 wt% Carbopol Aqua SF1, acetate buffer solution (pH 5.5) and each solution was sonicated for 5 minutes to facilitate its dispersing.

Determination of the berberine-loaded Carbopol nanogel particle size

The value of average particle diameter of berberine-loaded Carbopol Aqua SF1 was determined using the Malvern Zetasizer instrument and was found to be about 135 nm at pH 5.5 which corresponds to about 35% increase in particle size in comparison with bare carbopol (see figure 2) as shown in Figure S7. Figure S8 shows the zeta potential for the berberine-loaded Carbopol Aqua SF1 suspension and it can be seen that it was -40 mV. This high zeta potential of the berberine-loaded nanogel indicates that only a small fraction of COOH-groups on the nanogel matrix are bond to the loaded berberine and the COOH groups on the nanogel surface can dissociate almost to the same extent as bare Carbopol particles which ensures that the loaded carbopol suspension had high enough colloidal stability due to electrostatic stabilization. The TEM image shown in Figure S9 shows that the berberine-loaded Carbopol Aqua SF1 particles which have spherical morphology, although they are slightly larger than these of bare carbopol. The particle size on the image agrees with the results from Malvern Zetasizer (Figure S7).

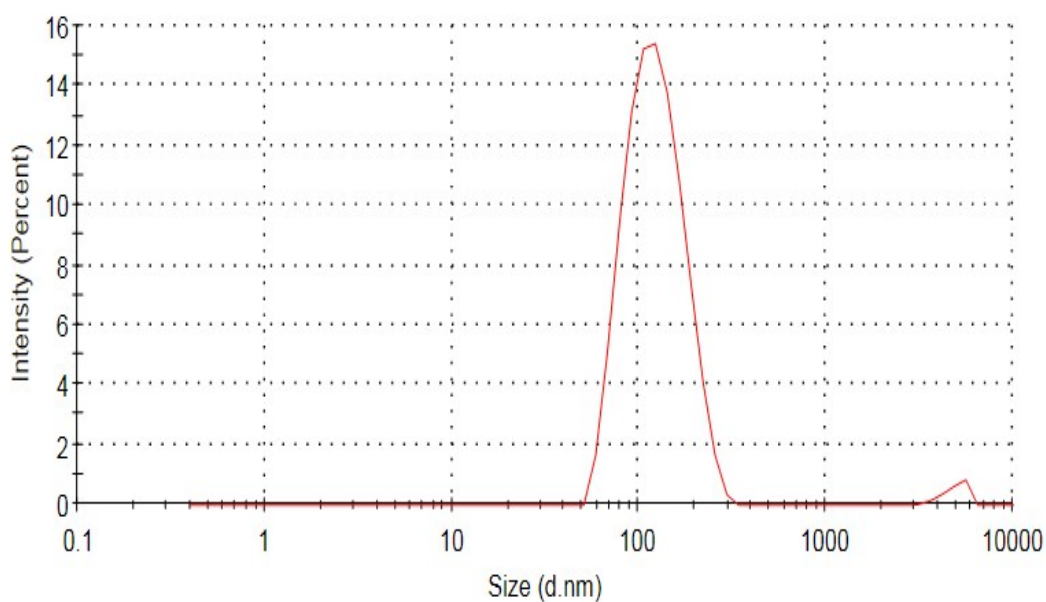


Figure S7. The Average particle diameter of 0.015 wt% berberine loaded Carbopol Aqua SF1 at pH 5.5 produced from addition of aliquot of berberine solution to 0.1 wt% of Carbopol Aqua SF1 Nanogel at pH 8 followed by decrease of the pH to 5.5 to form berberine-loaded collapsed Carbopol Aqua SF1. The final suspension was adjusted with acetate buffer solution supporting pH 5.5.

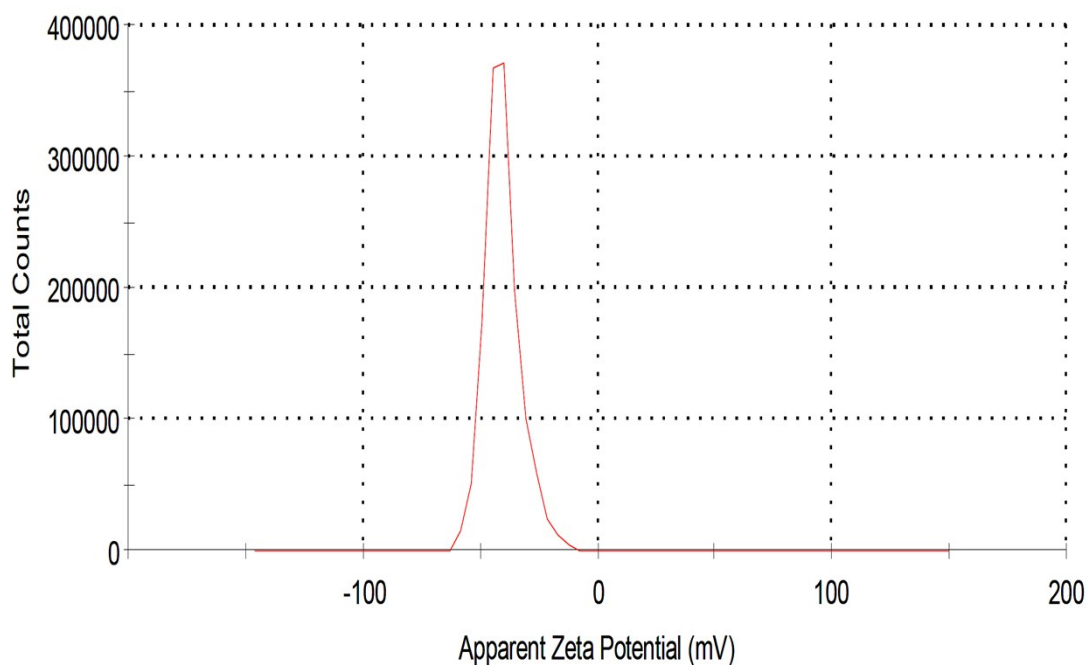


Figure S8. Zeta potential of 0.015 wt% berberine-loaded Carbopol Aqua SF1 at pH 5.5 acetate buffer solution which was measured by Malvern Zetasizer Nano ZS.

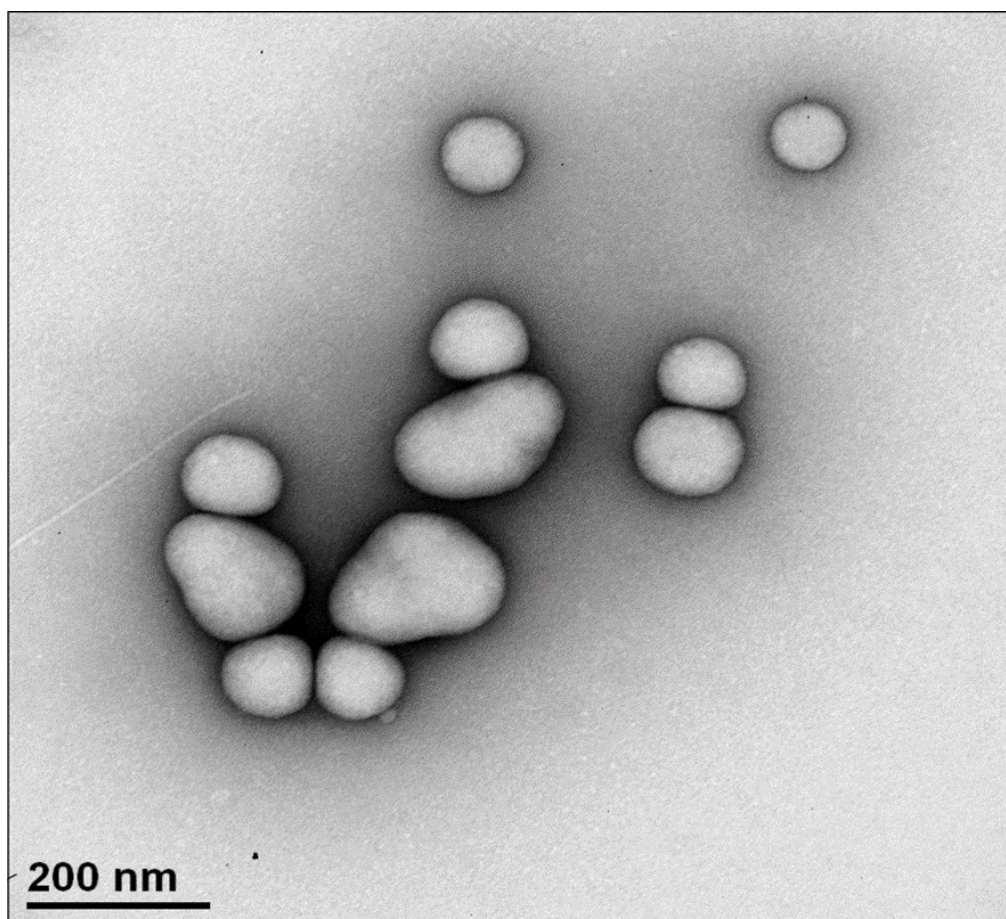


Figure S9. TEM image of berberine-loaded collapsed Carbopol Aqua SF1 at pH 5.5 adjusted with acetate buffer solution. The encapsulation was conducted through adjusting the nanogel at pH 8, then berberine was added to produce the swollen Carbopol Aqua SF1 nanogel. The produced suspension was adjusted to pH 5.5 to form berberine-loaded Carbopol Aqua SF1 nanogel.

Functionalization of Carbopol and berberine-loaded Carbopol nanogel

Solutions of a specific concentration of a range of cationic polyelectrolytes including poly (allylamine hydrochloride) (PAH), branched poly (diallyldimethylammonium) chloride (PDAC) and polyethyleneimine (PEI) were mixed with a fixed concentration of carbopol Aqua SF1 nanogel at a pH less than 5 to coat the nanogel with a layer of cationic polyelectrolyte. Figure S10 shows the average particle hydrodynamic diameter of the cationic polyelectrolyte coated Carbopol Aqua SF1 nanogels as a function of the type of cationic polyelectrolyte. PAH-coated and PDAC-coated Carbopol can be seen to have small particle diameters with a good stability in comparison with PEI which had much higher particle diameter (580 nm). PAH and PDAC were found to be the best cationic polyelectrolyte for the cationic functionalization of carbopol nanogel with high colloidal stability.

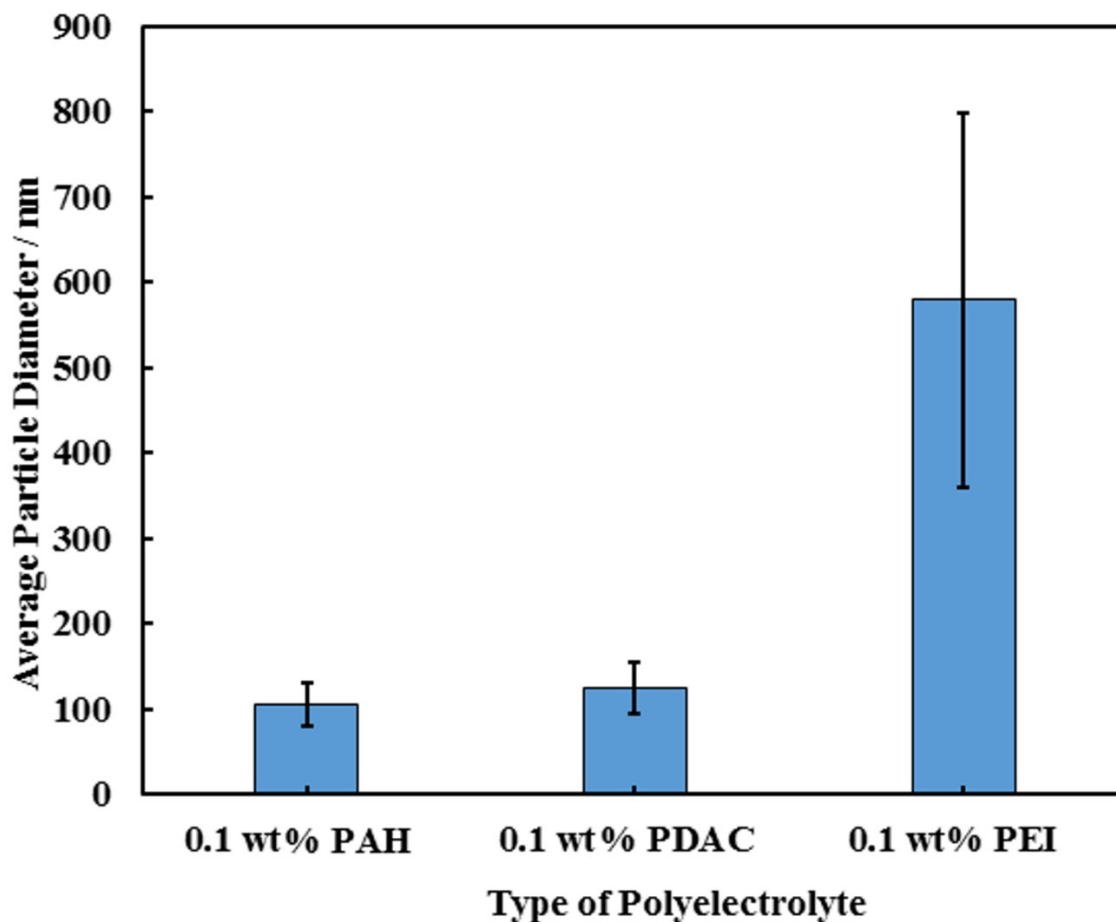


Figure S10. The average particle diameter of cationic polyelectrolyte functionalized Carbopol Aqua SF1 nanogel versus the category of cationic polyelectrolyte. The experiment was achieved by mixing excess amount of either poly (allylamine hydrochloride) (PAH), or branched poly (diallyldimethylammonium) chloride (PDAC) or polyethyleneimine (PEI) with 0.1 wt% Carbopol Aqua SF1 nanogel, then gently stirred and ultra-sonicated for a minute.

In order to incubate the cationic polyelectrolyte PAH-coated Carbopol Aqua SF1 nanogel with microorganisms, they needed to be washed to remove any PAH polyelectrolyte not coating the particles as the free PAH would have a cytotoxic effect on the microorganisms. The suspension was therefore centrifuged for an hour at 10,000 rpm twice and washed each time before measuring the average particle diameter at each time. After the first wash, the average particle diameter was 106 nm with a zeta potential around 30 mV as shown in Figure S11.

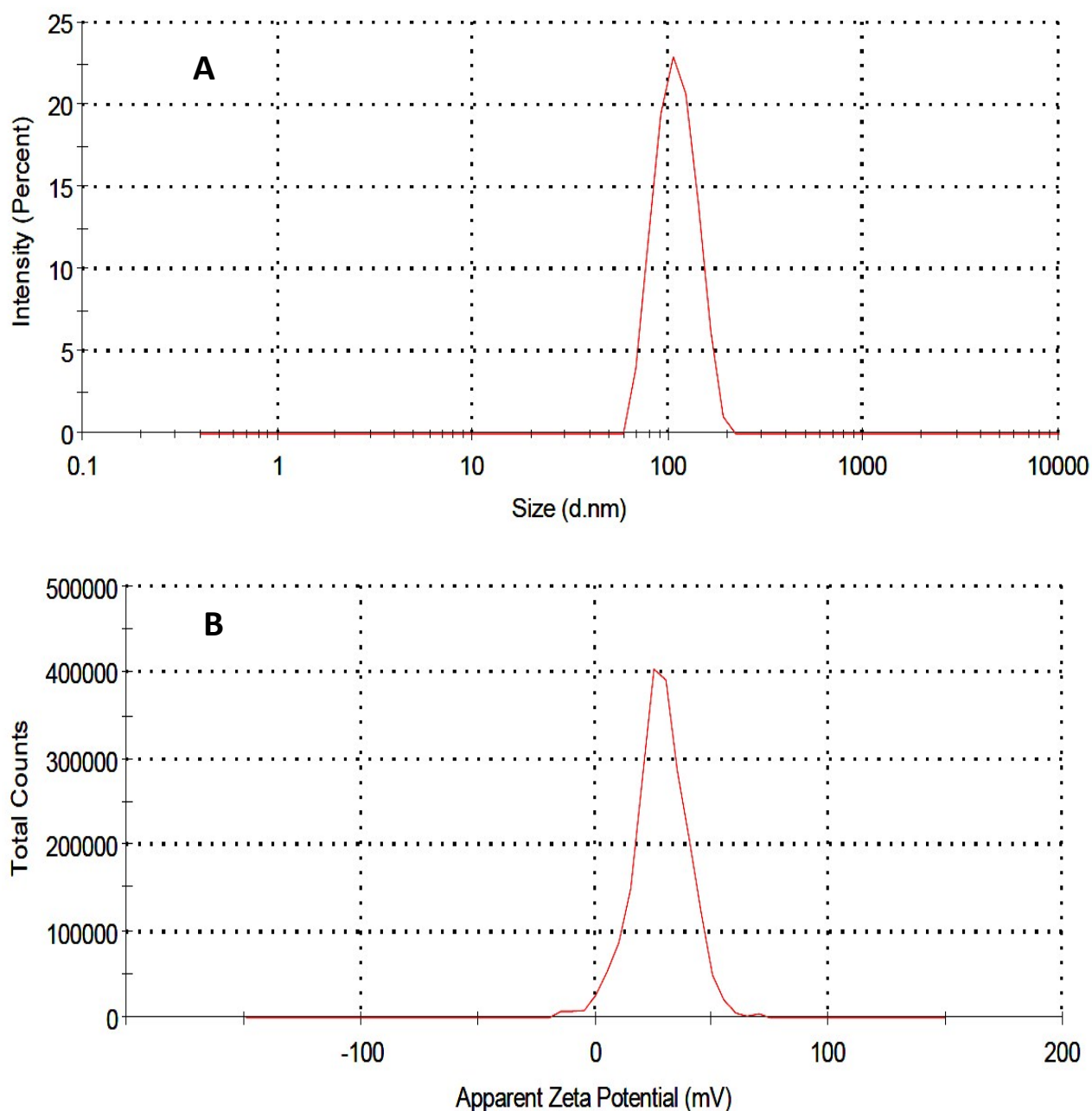


Figure S11. The average particle hydrodynamic diameter (A) and zeta potential (B) of PAH-coated Carbopol Aqua SF1 nanogel at pH 5. This was achieved through mixing excess amount of solution of the cationic polyelectrolyte (PAH) with a fixed concentration of Carbopol Aqua SF1 suspension to form PAH-coated Carbopol Aqua SF1. The produced suspensions were stirred, ultra-sonicated and centrifuged once at 10,000 rpm for 1 hour.

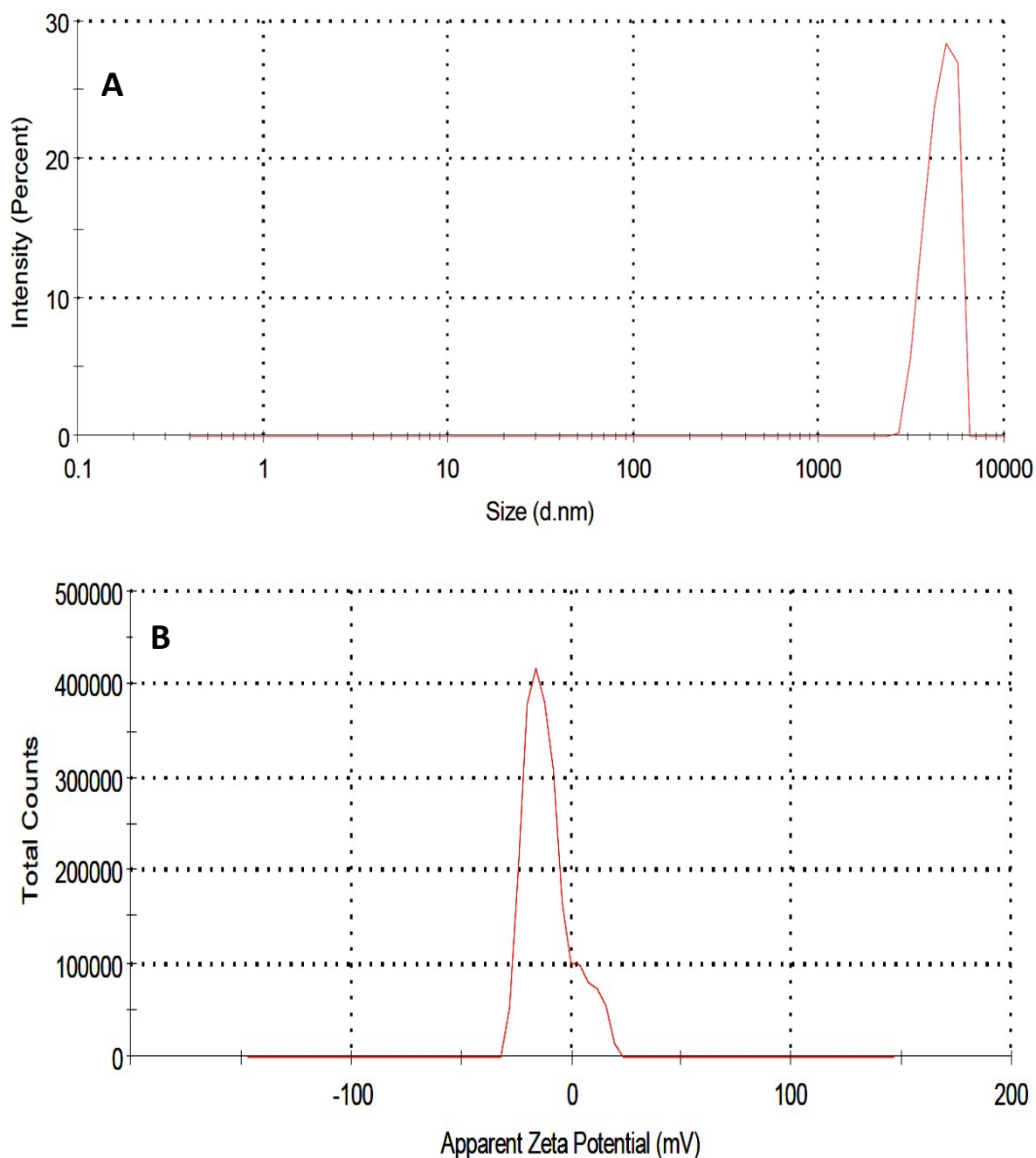


Figure S12. The average particle diameter (A) and zeta potential (B) of PAH-coated Carbopol nanogel at pH 5 which was conducted through stirring and ultra-sonicating of the suspension produced from the first centrifugation and then centrifuged again at 10,000 rpm for 1 hour.

Upon the second centrifugation, however as shown in Figure S12 that average particle diameter appeared too large at around 4 μm and the zeta potential was -11 mV which means that the PAH-coating had washed off the nanogel particle and was in the supernatant. Moreover, the coating of the nanogel particles was damaged which did not allow them to be re-dispersed in the solution.

Effect of the PDAC concentration on the Carbopol nanogel coating

The Carbopol Aqua SF1 nanogel was also coated with the cationic polyelectrolyte PDAC by mixing solutions of different concentrations of PDAC with a solution of fixed concentration of Carbopol nanogel to find a good balance between positive surface charge and colloidal stability of the PDAC-coated Carbopol nanogel. Figure S14 depicts the average particle hydrodynamic diameter and zeta potential of PDAC-functionalized Carbopol nanogel versus the PDAC concentration. Low concentrations of PDAC resulted in aggregation with low values for the zeta potential of around +12 mV while upon increase of the PDAC concentration up to 0.017 wt%, the average particle diameter and zeta potential of the PDAC-coated Carbopol were 228 nm and 15 mV, respectively. At 0.018 wt% PDAC concentration, the average particle size and zeta potential of Carbopol Aqua SF1 significantly decreased to 132 nm with a +18 mV. The particle diameter did not change for PDAC concentrations above 0.018 wt% but the zeta potential continued to increase to +44 mV at 0.1 wt% PDAC concentration. 0.018 wt% PDAC concentration was found to be the best compromise between positively charged nanogel and colloidal stability of the PDAC-coated Carbopol Aqua SF1.

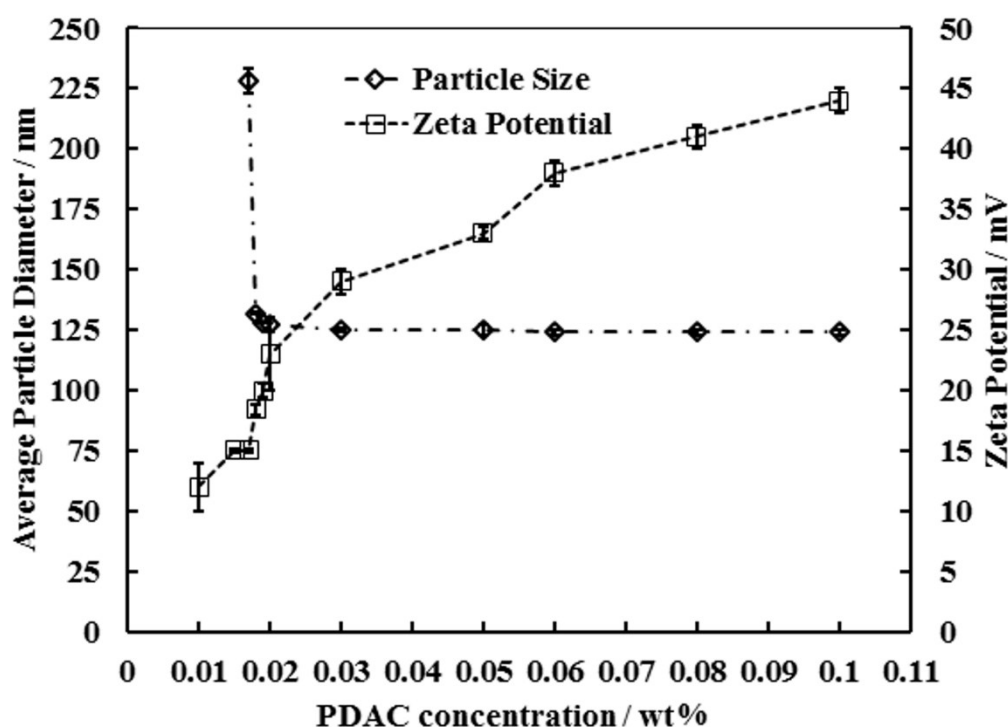


Figure S13. The average particle hydrodynamic diameter and zeta potential of PDAC-coated Carbopol Aqua SF1 suspension as a function of the PDAC concentration which were accomplished through mixing solutions of varied concentration of PDAC solutions with 0.1 wt% nanogel, followed by stirring and ultra-sonication for a minute. The particle diameter and zeta potential were measured by Malvern Zetasizer Nano ZS.

Cationic functionalization of antimicrobial-loaded Carbopol nanogel

PAH and PDAC are cationic polyelectrolytes which can kill microorganisms on their own through disrupting cell membranes.⁶ In addition, they can be utilized as coatings in promoting the adhesion of polyelectrolyte pre-coated particles to the cell membrane⁷ as well as to increase the antimicrobial activity of drug loaded nanoparticles.⁸ Both berberine-loaded Carbopol Aqua SF1 (BLC) were functionalized with PDAC to form PDAC-coated BLC. Figure S15 and Figure S16 show the particle size distribution and zeta potential for both PDAC coated BLC- and PDAC-coated CLC. The average particle diameter and zeta potential of PDAC coated BLC were 190 nm and +17 mV, respectively.

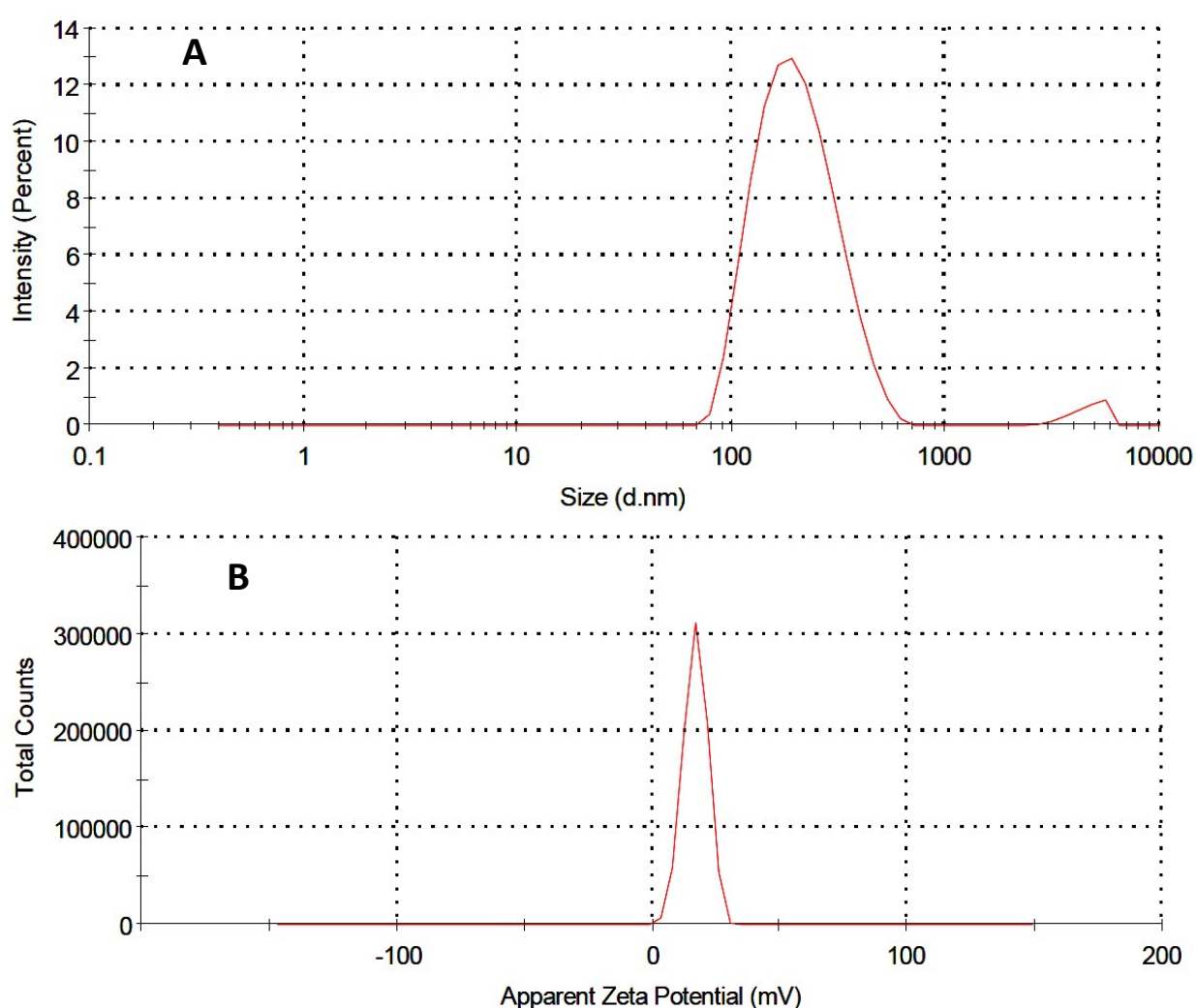


Figure S14. The particle size distribution (A) and zeta potential (B) of berberine-loaded carbopol nanogel particles coated with PDAC via addition of the PDAC solution of berberine-loaded carbopol nanogel, followed by shaking of the solution for 5 minutes.

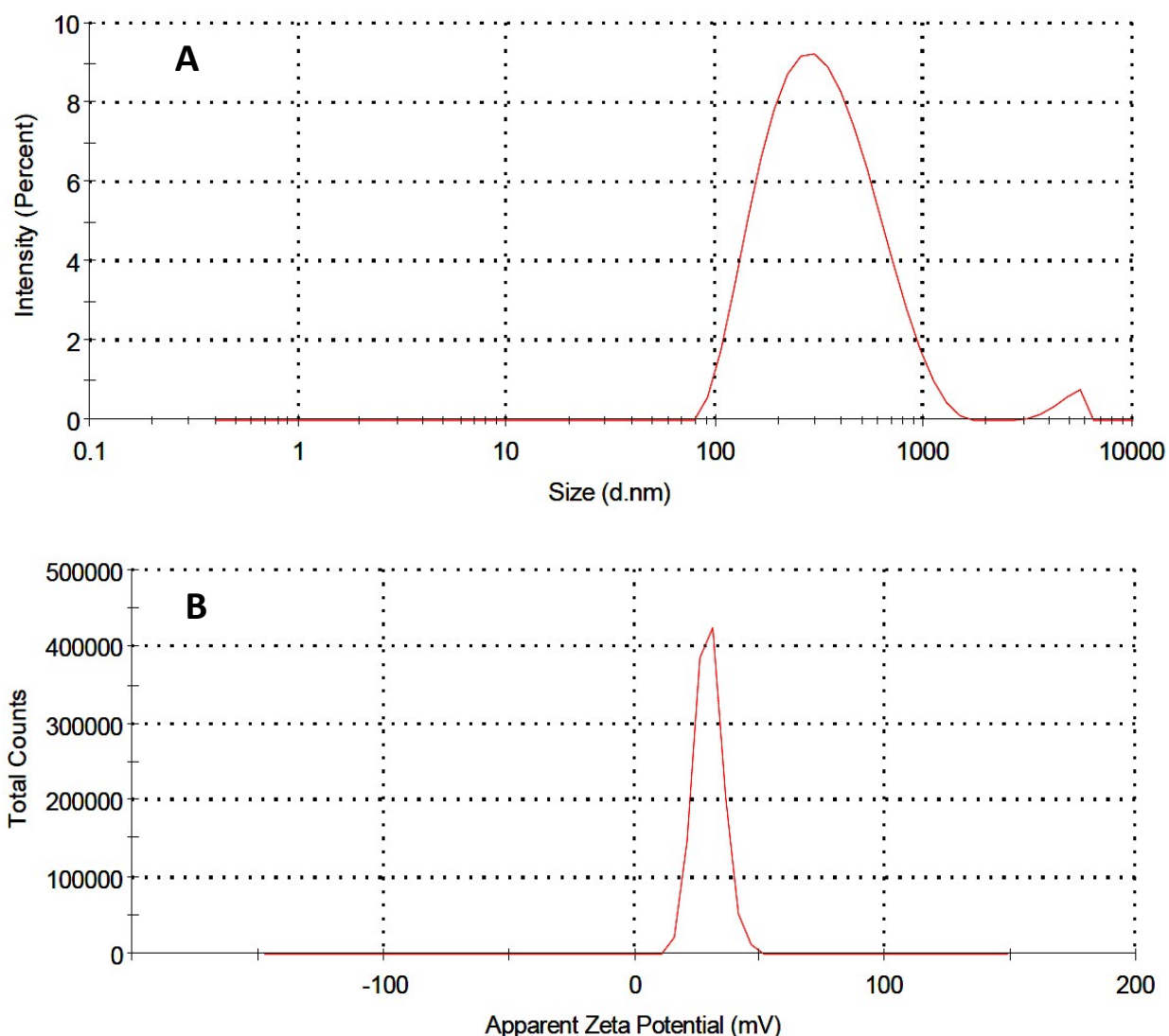


Figure S15. The particle size distribution (A) and zeta potential (B) of chlorhexidine-loaded carbopol coated with the cationic polyelectrolyte PDAC via addition of the cationic polyelectrolyte to the solution of Chlorhexidine-loaded carbopol, followed by shaking of the solution for 5 minutes.

It can be concluded that carbopol nanogel has two refractive index according to the change in pH where the refractive index is 1.45 in case of collapsed carbopol nanogel while it is 1.339 for the swelled nanogel. The results also indicated that carbopol nanogel can be used as a nanocarrier for drug delivery through encapsulation of berberine individually with high stability and improved drug release. It also indicated that the encapsulated berberine into carbopol nanogel has the ability to be coated with cationic polyelectrolyte such as PAH and PDAC to increase the antimicrobial actions.

UV-Visible spectrophotometry of berberine-loaded Carbopol

Carbopol, berberine, and berberine-loaded Carbopol Aqua SF1 were characterized by UV-vis spectrometry to examine the encapsulation of Berberine into Carbopol Aqua SF1 after all the excess berberine had been removed by the centrifugation/washing process which was carried out three times. The UV-visible spectrum of Carbopol Aqua SF1, berberine and berberine-loaded Carbopol Aqua SF1 is given in Figure S17. The Carbopol (blue line) absorbed in the UV region around 200 nm with no significant wavelength. The berberine (orange line) however had four absorption peaks, one which was in the visible area with a wavelength around 420 nm and three peaks in the UV region with wavelengths 350 nm, 265 nm and 230 nm respectively. It can be seen that there is no spectral interference between the absorbance of Carbopol Aqua SF1 and berberine. The black line shows the absorbance spectrum for the berberine-loaded Carbopol Aqua SF1 and the spectra for berberine can be clearly seen which showed four peaks similar to those of Berberine. As the all the excess berberine had been removed by the centrifugation process, this provides clear evidence that the cationic berberine is conjugated with the anionic carboxyl group of the Carbopol nanogel.

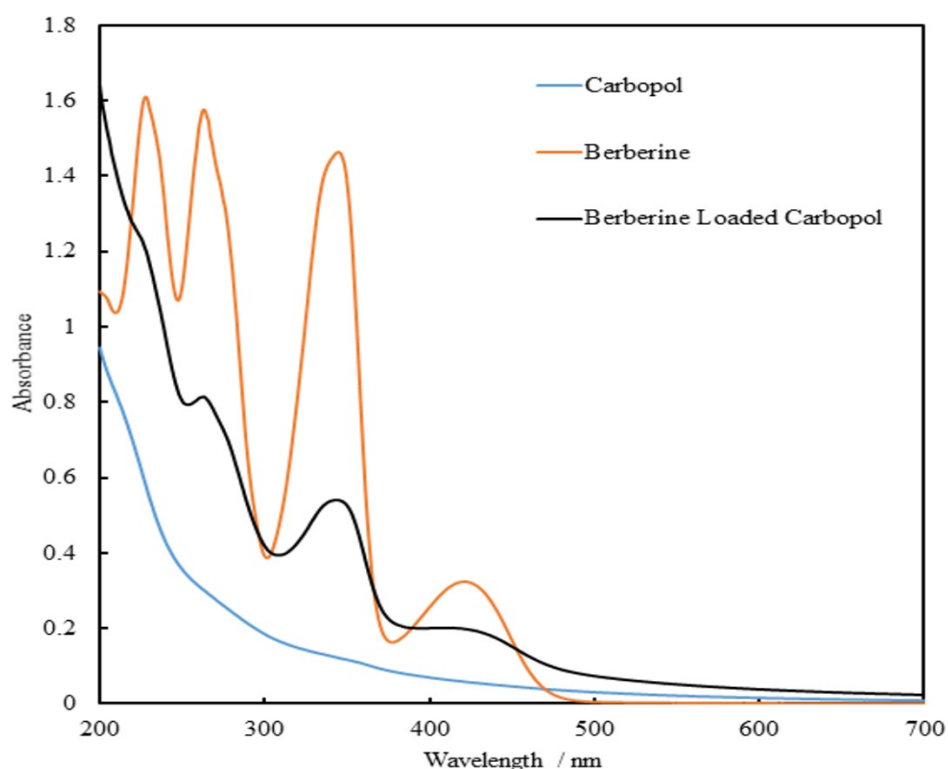


Figure S16. The UV-Visible spectrum of 0.01 wt% carbopol (Blue line), 0.025 wt% Berberine (Orange line) and berberine-loaded carbopol (Black line). All solutions were adjusted to pH 5.5 using acetate buffer solution. Perkin Elmer Lambda 25 UV-Visible spectrophotometer was utilized to measure the spectrum in the wavelength range from 200 nm to 700 nm.

FTIR spectrometry of berberine loaded Carbopol

Fourier Transform Infra-Red spectroscopy (FTIR) was used to further explore the composition of the loaded carbopol particles. Spectra were obtained for the Carbopol nanogel, berberine and berberine-loaded Carbopol nanogel in the frequency range from 400 cm^{-1} to 4000 cm^{-1} . In the FTIR spectra of Carbopol nanogel as shown in Figure S18A, a characteristic peak was seen in the range $3300\text{--}3500\text{ cm}^{-1}$ due to the stretching vibrations of O-H and intermolecular hydrogen bonding. The strong peak between 2850 and 3000 cm^{-1} was due to the stretching vibration band of the aliphatic C-H bond. The spectra also presented a peak in the range $1670\text{--}1750\text{ cm}^{-1}$ which appointed to a strong vibration band of carbonyl stretching (C=O) while the two variable peaks at $1400\text{--}1490\text{ cm}^{-1}$ were assigned to the stretching vibration band of the carbonyl bond (C-O). The peak appearing from 1000 cm^{-1} to 1300 cm^{-1} was attributed to the coupling between in-plane OH bending and C-O stretching of neighboring carboxyl groups. These explanations of FTIR spectrum of Carbopol Aqua SF1 are in line with the literature.^{9,10}

For berberine, it can be seen in Figure S18B that two weak peaks were noticeable at 2946 cm^{-1} and 2846 cm^{-1} which are assigned to the stretching vibration of the aliphatic C-H group. It was also observed significant bands of berberine at 1636 cm^{-1} , 1602 cm^{-1} and 1505 cm^{-1} and these bands are belonging to heterocyclic amine (C-N), quaternary iminium ion (-C=N-) and the stretching vibration of C=C in the aromatic ring, respectively. However, a peak at 1105 cm^{-1} was prominent which was attributed to the deformation of aromatic ring and the bending vibration in C-H in-plane. In addition of that, the stretching vibration of C-H was presented at 1037 cm^{-1} . The FTIR results of berberine are in line with the literature.¹¹⁻¹³

The FTIR spectrum of berberine-loaded Carbopol Aqua SF1 are shown in Figure S18C which illustrated some characteristic bands for the encapsulation of berberine into Carbopol Aqua SF1. A broad vibration band at 3460 cm^{-1} was referred to stretching of the O-H bond which was also noticed with the FTIR of Carbopol. Furthermore, it was perceived that a peak at 1700 cm^{-1} is related to the stretching vibration band of carbonyl group (C=O) of the Carbopol (PAA) while another two distinctive peaks were prominent in the berberine-loaded Carbopol at 1653 cm^{-1} and 1600 cm^{-1} for the stretching vibration of C-N and -C=N- bands. These bands were also noticed in the berberine only. Finally, a significant peak at 1500 cm^{-1} was noted that represents the stretching vibration band of C=C for the aromatic ring.

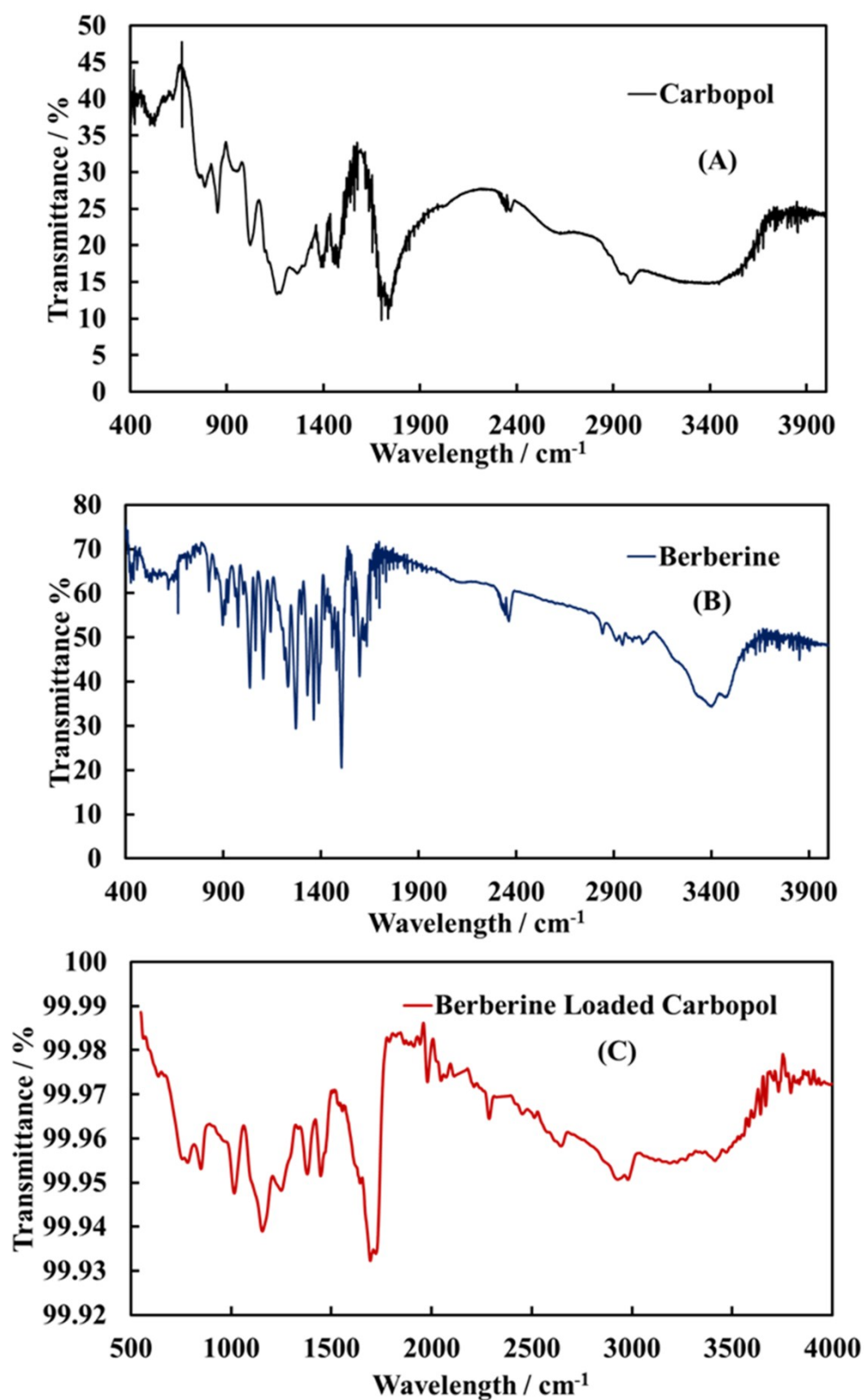


Figure S17. The FTIR spectrum of Carbopol Aqua SF1 (A), berberine (B) and berberine-loaded Carbopol Aqua SF1 (C). Each sample was dried overnight and mixed with excess amount of dried KBr pellets to make KBr disk. The samples then scanned with Perkin Elmer FT-IR spectrometer in the range of wavelength from 400 cm^{-1} to 4000 cm^{-1} .

Thermogravimetric analysis of berberine-loaded Carbopol

The thermal gravimetric analysis was used to study the physical structures of berberine, Carbopol and berberine-loaded Carbopol nanogel. The thermal gravimetric analysis can be seen in Figure S19 along with the 1st derivative curve (DTA). The materials were heated from 100 °C to 900 °C at 10 °C/minute in air atmosphere with a purge rate of 10 mL/minute. Carbopol (C) (blue curve) lost weight at 400 °C which relates to the thermal decomposition of hydrocarbon compounds, no changes were seen over 550 °C. The berberine component in Figure S19 (black curve) gave an endothermic peak at 150 °C which was attributed to loss of water. After that, upon increasing the temperature little change was seen up to 700 °C. The thermal behavior of the berberine loaded carbopol was different to that for the individual compounds (red Curve) The TGA curve showed weight loss at 300 °C, 350 °C and 450 °C.

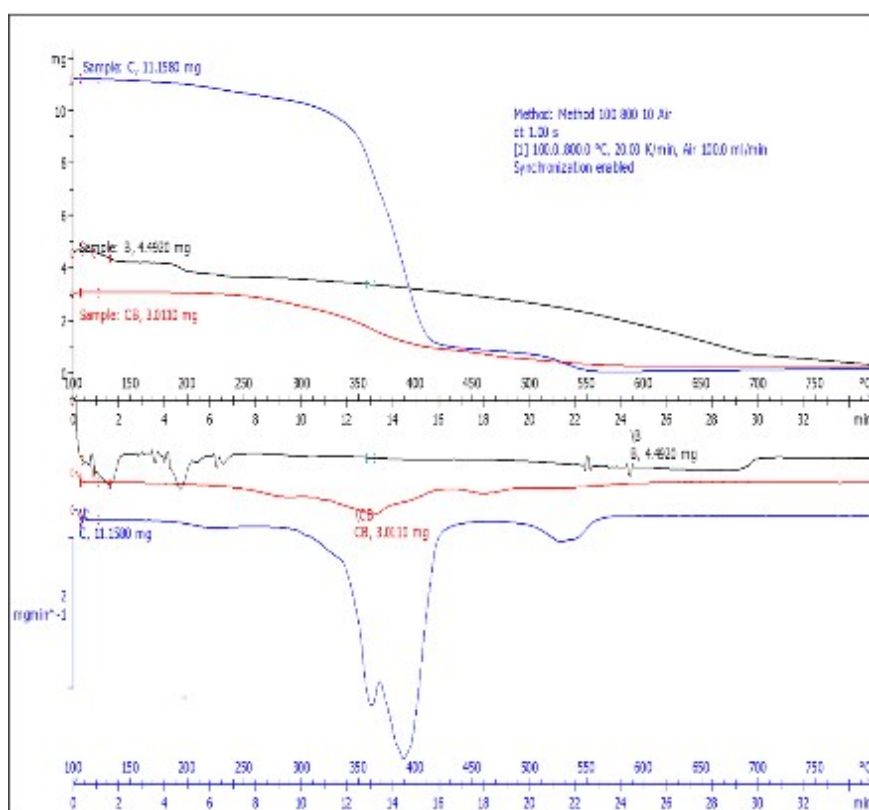


Figure S18. The thermogravimetric analysis and the first derivative curve of Carbopol (Blue curve), berberine (Black curve) and berberine-loaded Carbopol (Red curve) where the mass is plotted in the Y-axis while the temperature on the X-axis. This experiment was conducted in the air atmosphere with a purge rate of 10 mL/minute at temperature from 100 °C to 900 °C.

Elemental analysis of berberine-loaded Carbopol Aqua SF1 nanogel

The elemental analysis of Carbopol alone and for the encapsulated berberine was conducted by taking an appropriated amount of 0.15 wt% berberine-loaded 0.1 wt% Carbopol Aqua SF1 nanogel. The nanogel particles was centrifuged, dried and vacuumed overnight at room temperature. The precipitates formed were studied by elemental analysis. Table S1 shows the % value of C, H, and N for Carbopol and berberine-loaded Carbopol. Carbopol contained a higher percentage of carbon in comparison with hydrogen amount but there was no nitrogen in carbopol content which means that Carbopol is typical cross-linked polyacrylic acid derivative consisting of two main elements, carbon and hydrogen in addition to the oxygen which was not analyzed. However, the berberine-loaded Carbopol nanogel as expected had carbon, hydrogen and nitrogen demonstrating that the berberine had been encapsulated into the Carbopol particles. Using the percentages of each elements resulted from the elementary analysis, the composition of the loaded particles was determined to contain 12.2 % berberine and 87.8 % carbopol. Therefore, if the dry carbopol is assumed to have the same composition as polyacrylic acid, the mole ratio of the carboxyl group with respect to the loaded berberine is about 33:1.

Table.S1: The elemental analysis of Carbopol SF1-Aqua and berberine-loaded Carbopol Aqua SF1 nanogel. The berberine content in the nanogel particles is calculated based on their nitrogen content. The mole ratio between the COOH groups of the carbopol and the encapsulated berberine is estimated based on carbon and nitrogen content of the berberine-loaded Carbopol Aqua SF1.

Element	Carbopol %	Berberine-loaded Carbopol %
C	56.8	54.7
H	5.0	4.0
N	0.0	0.51
% Berberine content in Carbopol		12 %
Mole Ratio (COOH groups / Berberine)*		33.5

Berberine encapsulation efficiency and loading content in Carbopol SF1 nanogel

The supernatant produced from the encapsulation of drug into Carbopol Aqua SF1 was taken and the absorbance was measured at 430 nm for berberine using UV-Visible spectrophotometer. Calibration curves of each drug were created by preparing different concentrations of the drug and measuring the absorbance of each concentration. Then, a graph was plotted for the absorbance as a function of the concentration of the drug. The linear regression equation was used to calculate the unknown concentration of the unencapsulated drug. The encapsulation efficiency of the active material loaded Carbopol Aqua SF1 was calculated by the equations

$$\text{Encapsulation Efficiency (\%)} = \frac{\text{Total Drug Amount} - \text{Unencapsulated Drug Amount}}{\text{Total Drug Amount}} \times 100$$

$$\text{Drug Loading Content (\%)} = \frac{\text{Total Drug Amount} - \text{Unencapsulated Drug Amount}}{\text{Total Drug Amount} - \text{Unencapsulated Drug Amount} + \text{Carbopol amount}} \times 100$$

The non-encapsulated amount of free berberine in the nanogel suspension was measured to determine the encapsulation efficiency and the percentage of the berberine loading content. This was done by a linear regression equation from the calibration curve of berberine. Table S2 shows the encapsulation efficiency and the percentage of berberine loading content of the added concentration of berberine. It can be proved that the encapsulation efficiency of berberine into Carbopol nanogel is around 10% as berberine contains one ionisable amino group that can interact electrostatically with each deprotonated carboxyl groups in the Carbopol nanogel at pH 5.5. Additionally, the percentage of berberine loading content is 9% which represents the amount of loaded berberine with respect to the amount of dried carbopol nanogel (from initial 0.1 wt% concentration). The following equations were used to calculate the encapsulation efficiency and drug loading content of Berberine (BRB):¹⁴

$$\text{Berberine Encapsulation (\%)} = \frac{[w_{BRB} - w_U]}{w_{BRB}} \times 100, \quad (15)$$

$$\text{Drug Loading Content (\%)} = \frac{[w_{BRB} - w_U]}{[w_{BRB} - w_U + w_C]} \times 100. \quad (16)$$

Here w_{BRB} is the total amount of Berberine added to the carbopol at pH 8, w_U is the non-encapsulated amount of Berberine obtained after the centrifugation and analyzing the supernatant, and w_C is the amount of Carbopol used during the whole system which means the concentration of Carbopol nanogel in wt%.

Berberine release from berberine-loaded Carbopol nanogel suspensions

A 50 ml of berberine-loaded 0.1 wt% Carbopol Aqua SF1 was added into a dialysis bag (10K MWCO). Then, the bag was placed into 500 ml beaker which has already being filled with 500 ml of acetate buffer solution (pH 5.5) (1M: 1M Acetic acid: Sodium Acetate) and capped with an aluminum foil. The bag was stirred gently with an orbital shaker at room temperature. After that, the drug release was measured in a range of wavelength from 400 nm to 200 nm using UV-Visible spectrophotometer of buffer solution in the micro centrifuge tube against water sample as a blank. The measurements were taken at 15, 30, 60, 120, 180, 240, 300, 360 and 1440 minutes. All release experiments were carried out in triplicate. The percentage of cumulative drug release can be calculated by the formula

$$\% \text{ In Vitro Drug Release} = \frac{m_R}{m_L} \times 100$$

where m_R is the amount of drug released from the Carbopol Aqua SF1 at time t and m_L is the amount of drug loaded in the Carbopol Aqua SF1.

Table S2: The encapsulation efficiency and the percentage of berberine loading content of 0.15 wt% berberine (BRB) into 0.1 wt% of Carbopol nanogel.

Total BRB concentration wt%	BRB Non-encapsulated wt%	BRB encapsulated wt%	Encapsulation efficiency %	Drug loading content %
0.15	0.135 ± 0.008	0.015 ± 0.008	10 ± 6	9 ± 5

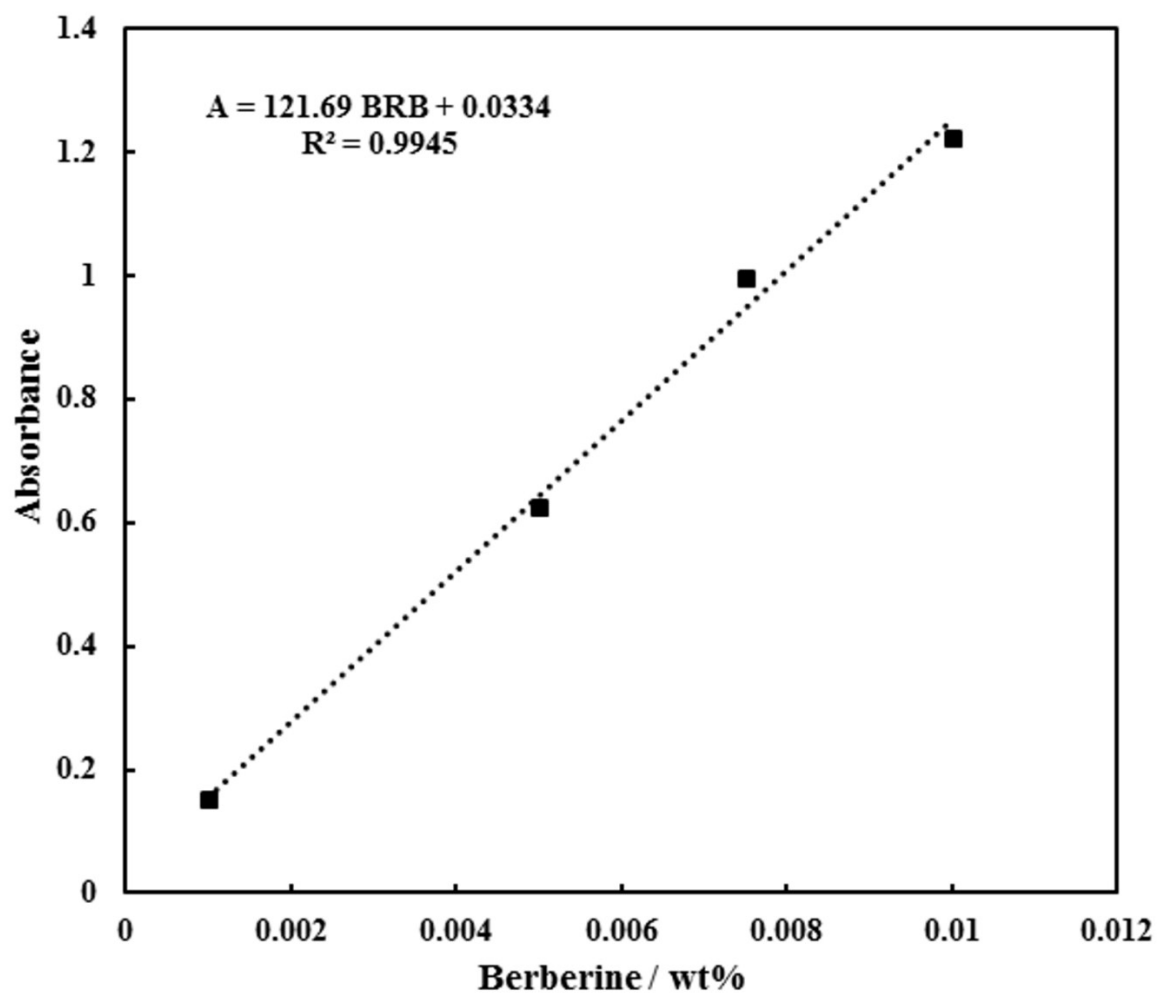


Figure S19. The absorbance vs concentration of series of solutions of berberine concentrations measured at 422 nm wavelength. The diluted concentrations of berberine were prepared from 0.2 wt% stock solution of berberine chloride.

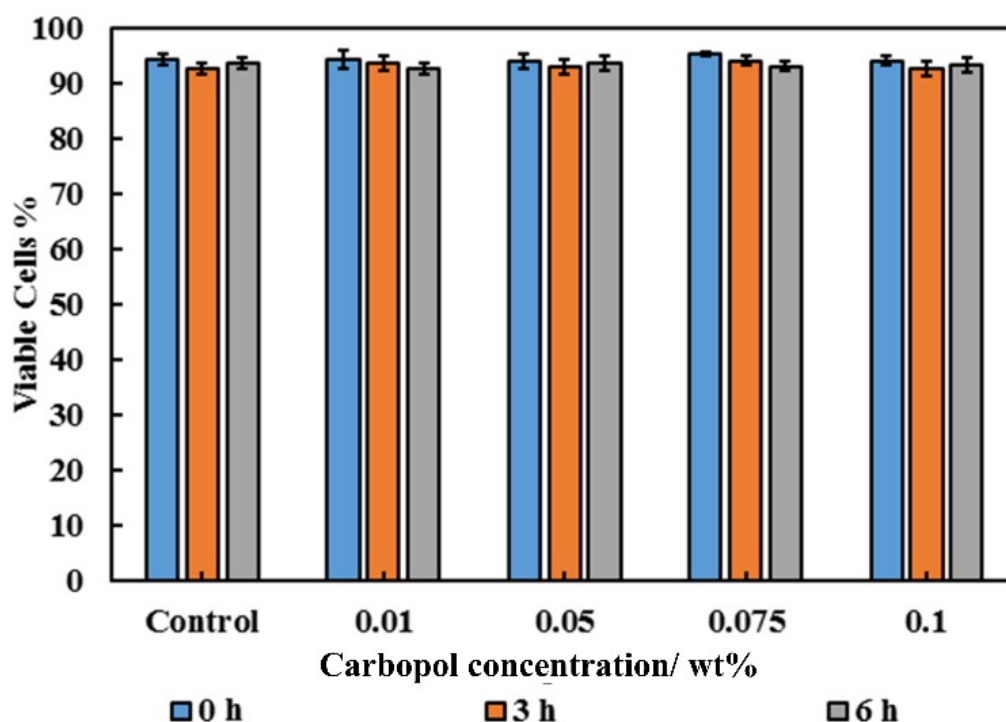


Figure S20: The cell viability of *C.reinhardtii* incubated in aqueous suspensions of varying concentrations of Carbopol Aqua SF1 nanogel particles.

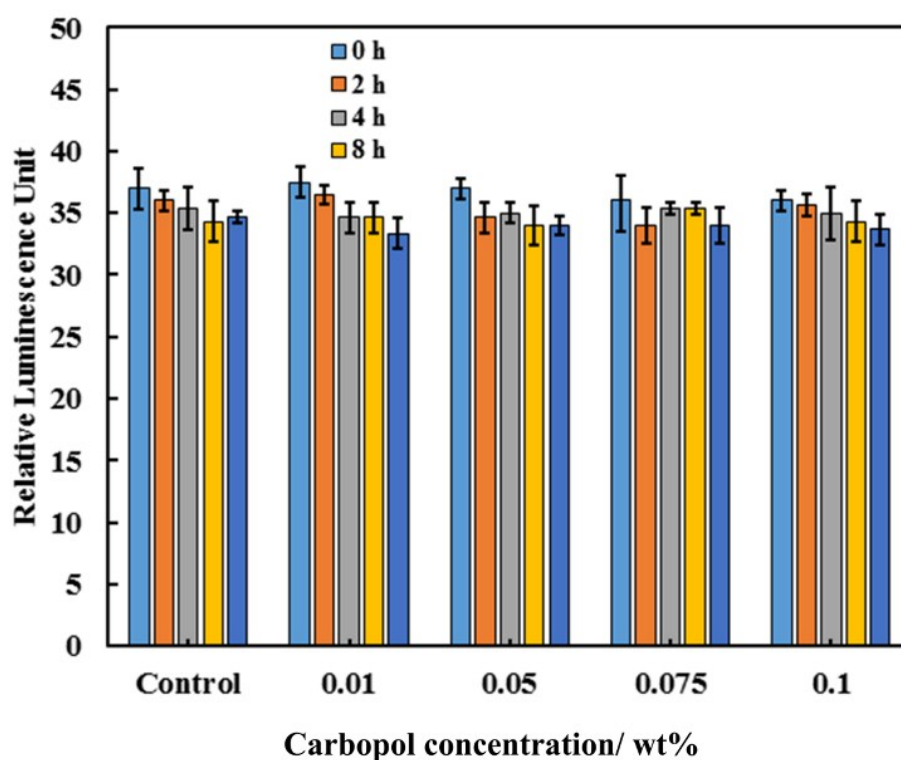


Figure S21. Cytotoxic effect of suspensions of different concentration of Carbopol Aqua SF1 nanogel particles on *E.coli* at different incubation times. The experiment was conducted by incubation of the cells with the nanogel suspension at pH 5.5 at room temperature for up to 24 hours.

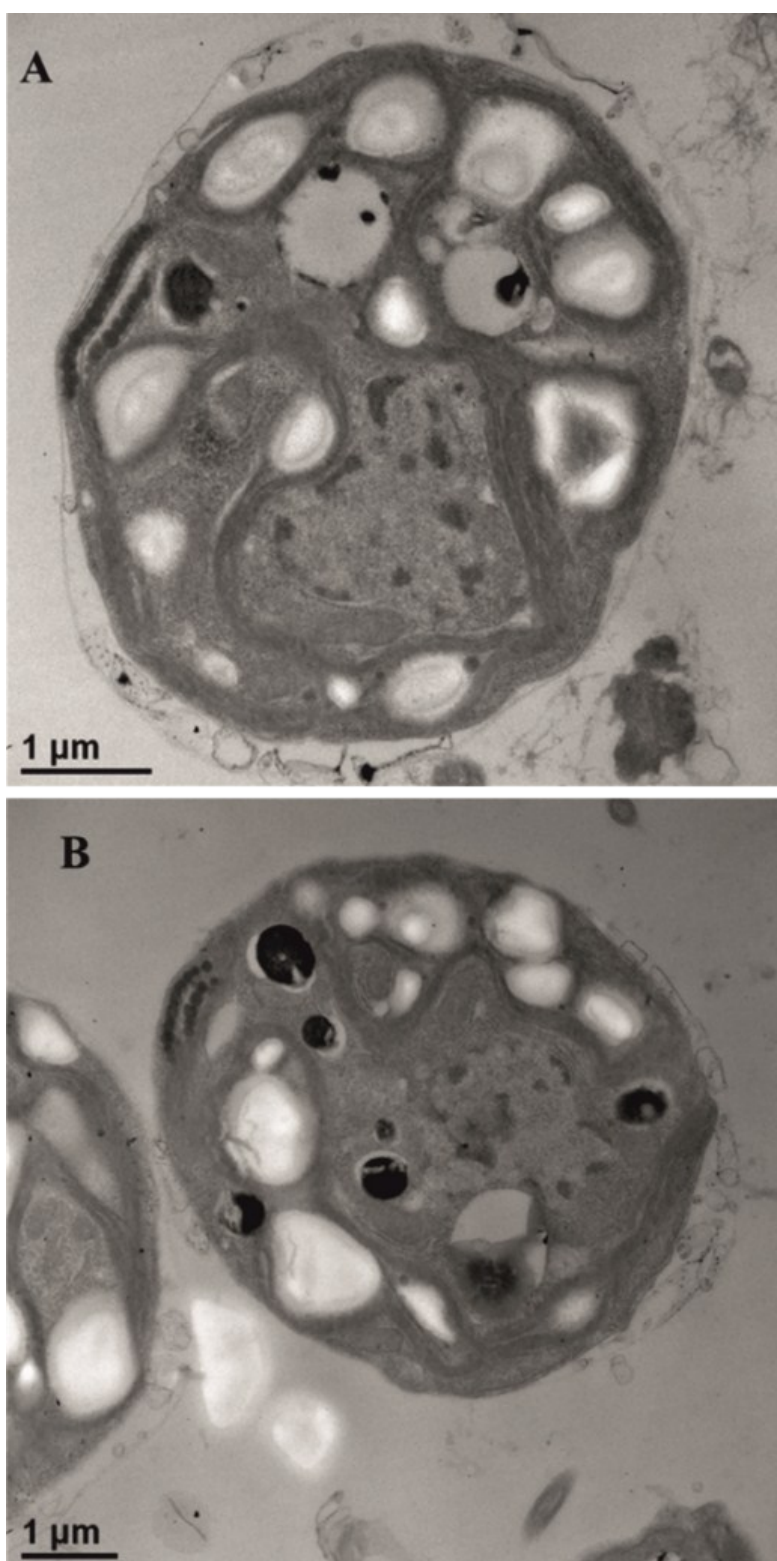


Figure S22. The TEM images of the *C. reinhardtii* cells: (A) before and (B) after incubation with 0.1 wt% Carbopol Aqua SF1 nanogel dispersion.

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