Application Note

Pharmaceuticals

Agilent

Trusted Answers

Parallel Synthesis and Characterization of a Chitosan Drug Carrier System

Cary 3500 Peltier UV-Vis spectrophotometer used to monitor cross-linking mechanism

Author

Dr. Mohammad Al Kobaisi[1]

Dr. Matthew Quinn[2]

[1]Swinburne University of Technology, School of Science, Faculty of Science Engineering and Technology, Melbourne, Australia

[2]Agilent Technologies, Inc. Melbourne, Australia

Introduction

Stable, controllable, and biocompatible drug carrier systems are of high importance and can have significant impacts in drug and vaccine delivery systems (*1, 2*). Finding novel and improved methods of preparing drug carrier systems can avoid the use of less biocompatible materials and provide more stable carriers, as well as easier routes of preparation (*3*).

Chitosan is a polysaccharide present in the shells of crustaceans such as crabs and lobsters. It was selected for this study as it has been explored extensively due to its highly robust chemical activity, biocompatibility, and capacity for controlled release (*1, 4, 5*). Chitosan dissolves in weak acidic aqueous solvents and can be covalently or ionically cross-linked to form hydrogel devices with various sizes and shapes. Polyphosphates are commonly used to produce nano- and micro-particles of chitosan. Here we study a new polyphosphate to induce chitosan hydrogelation; octaphosphonate tetraphenyl porphyrin (OPP) is a photoactive cromophor molecule

that can be utilized as a chitosan cross-linker (Figure 1). The cross-linking process between chitosan and OPP results in the self-assembly of OPP chromophores. The mechanism causes spectroscopic changes that can be monitored using UV-Vis spectroscopy.

The phosphonate groups of OPP (Figure 1) in their anionic form interact strongly with the protonated amines of chitosan to cross-link polymer chains in solution. This process results in the formation of particles approximately 1 µm in diameter, capable of encapsulating a wide range of biologically active compounds such as vaccine antigens or drugs.

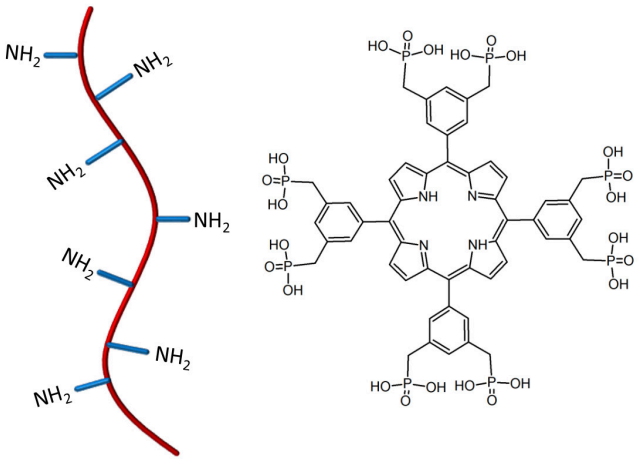

Figure 1. (Left) Schematic representation of chitosan with available amine groups and (right) the molecular structure of octaphosphonate tetraphenyl porphyrin (OPP) showing the available phosphonate groups.

In this study, we investigated the OPP-chitosan cross-linking mechanism, which was monitored using an Agilent Cary 3500 UV-Vis spectrophotometer. The Cary 3500 is particularly suited to this work, with its software-controlled in-cuvette stirring functionality and insensitivity to ambient light. The latter allows reagents to be sequentially added without impacting the data in a time-based measurement. The number of steps is minimized by performing the synthesis *in situ*.

Experimental

Chitosan-FITC-OPP micro-hydrogel preparation and monitoring

Fluorescein isothiocyanate (FITC) labeled chitosan solution in 1 % acetic acid was used in this experiment. The samples were prepared by gradual addition of chitosan solution to a fixed volume of OPP solution via the following setup:

1. 2 mL of 0.125 mg/mL of OPP with the pH set to approximately 7 was added to a 3.5 mL quartz cuvette, fitted with a star type PTFE magnetic stirrer bar.
2. A cuvette of water was placed in the reference channel as a control.
3. The pH of the OPP solution was then adjusted to 4.5 using 1 N hydrochloric acid (HCl) to match the pH of the chitosan solution. This ensured that the observed changes during the cross-linking process could not be attributed to pH changes.
4. A wavelength scan was performed after a 20 second equilibration period.
5. A series of small volume additions of 2 % w/v chitosan-fluorescein isothiocyanate (FITC) were added as per Table 1. After each addition, a wavelength scan was performed following a 20 second equilibration period.
6. At the end of the chitosan-FITC additions, the pH was returned to 7 with the introduction of 1 N sodium hydroxide (NaOH). A further wavelength scan was performed after a 20 second equilibration period.

Table 1. Additions made to the sample during the experiment.

Exp. #	Vol Chitosan (µL)	Vol Total (µL)	Chitosan (mg/mL)	OPP (mg/mL)	pH
1	0	1500	0.000	0.125	7.0
2	0	1500	0.000	0.125	4.5
3	20	1520	0.263	0.123	4.5
5	40	1540	0.519	0.122	4.5
6	90	1590	1.132	0.118	4.5
7	140	1640	1.707	0.114	4.5
8	200	1700	2.353	0.110	4.5
9	300	1800	3.333	0.104	4.5
10	500	2000	5.000	0.094	4.5
11	700	2200	6.364	0.085	4.5
12	1000	2500	8.000	0.075	4.5
13	1500	3000	10.000	0.063	4.5
14					4.5
15					5.0
16					5.4
17					6.0
18					6.5
19	1500	3000	10.000	0.063	7
20	1500	1500	20.000	0	4.5

Instrumentation

An Agilent Cary 3500 Compact UV-Vis spectrophotometer was selected for this study. The instrument parameters are shown in Table 2.

Table 2. UV-Vis experimental parameters.

Parameter	Setting
Wavelength Range (nm)	1100 to 200
Spectral Bandwidth (nm)	1
Signal Averaging Time (s)	0.1
Data Interval (nm)	1
Stirring Speed (rpm)	500

Results and discussion

Preparation of particles

The preparation of the OPP cross-linked chitosan was monitored by following the primary OPP peak at 417 nm at pH 7 and 441 nm at pH 4.5, as shown in Figure 2. The pH was shifted from 7 to 4.5 to avoid changes in pH upon the addition of the acidic chitosan precursor solution. Changing the pH was important as the absorbance of OPP is pH dependent.

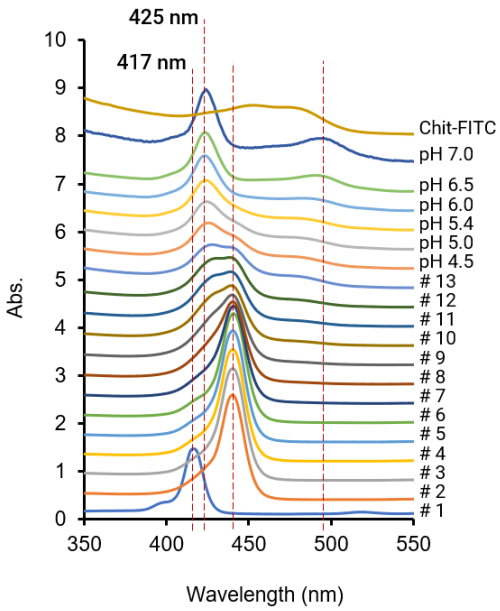

Figure 2. Wavelength scans (350 to 550 nm) of chitosan-FITC during cross-linking process using OPP. The numeric labels #1 to #13 are the experiment numbers, shown in Table 1. Spectra are offset for clarity. The peak at 425 nm is indicative of OPP, associated with the cross-linked chitosan-FITC.

Following the adjustment of pH to 4.5, the chitosan precursor was gradually added. This showed little effect on the OPP 417 nm peak until the chitosan-FITC concentration reached 0.261 % w/w, whereby the emergence of a peak at 425 nm was observed.

The solution was then incrementally adjusted back to the physiologically relevant pH of 7, which is the OPP starting pH. The spectrum of the product solution, adjusted to pH 7, showed a clear peak at 425 nm, and no presence of the original peak at 417 nm. The absorbance at 425 nm can be assigned to the OPP, associated with the cross-linked chitosan-FITC only. The presence of the cross-linked chitosan particles is also indicated by the increased signal across the wavelength range (increasing with decreasing wavelength) indicative of light scattering. This shows more clearly at lower wavelengths (Raleigh scattering) and can be easily observed at approximately 300 nm, as shown in Figure 3.

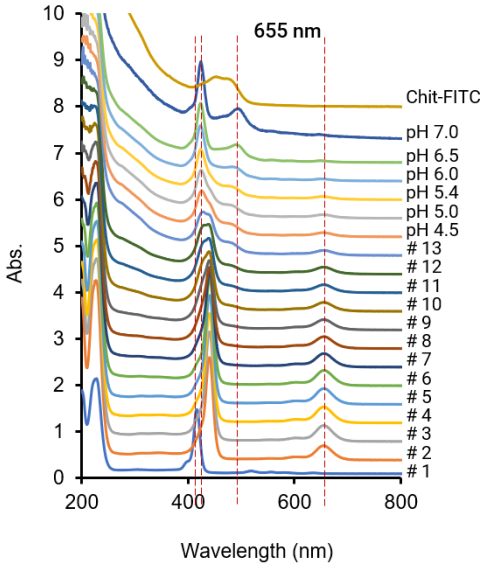

Figure 3. Wavelength scans (200 to 800 nm) of chitosan-FITC during cross-linking process using OPP. The numeric labels #1 to #13 are the experiment numbers, shown in Table 1. Spectra are offset for clarity. The increased signal at the lower wavelengths indicates particle scattering. The peak at 655 nm is indicative of free OPP.

Free OPP absorbs at approximately 655 nm. By monitoring the 655 nm peak, the depletion of OPP could be determined (see Figure 3). This demonstrated that the process exhibited a titration behavior, where an equivalent point can be observed, and the reaction end-point can be detected.

The wavelength scans of the polymer without any OPP confirmed that the spectral peak shift changes and absorbance intensity changes are due to the change in the environment of the OPP chromophore. These spectral changes can be attributed to the variation in the electrostatic environment and the proximity of the aromatic cores that are enhanced by the aggregation of the molecules during the cross-linking process (Figure 2).

Conclusion

A Cary 3500 Compact UV-Vis spectrophotometer was used to monitor the synthesis of a novel biopolymer drug carrier system, a chitosan-FITC-OPP micro-hydrogel. The software controlled stirring functionality of the 3500 allowed the synthesis to be performed with simple additions directly into the cuvette. By continually monitoring the synthesis by performing wavelength scans, the point at which the neat cross-linker absorbance peak shifted from 417 to 425 nm could be determined. The peak shift indicated that cross-linking had occurred and that the micro-hydrogel particles had formed.

References

1. Mohammed, M. A.; Syeda, J. T. M.; Wasan, K. M.; Wasan, E. K. An Overview of Chitosan Nanoparticles and Its Application in Non-Parenteral Drug Delivery. *Pharmaceutics,* **2017**, 9 (4), 53.
2. Bernkop-Schnürch, A.; Dünnhaupt, S. Chitosan-based drug delivery systems. *Eur. J. Pharm. Biopharm.,* **2012**, 81 (3), 463.
3. Hu, L.; Sun, Y.; Wu, Y. Advances in chitosan-based drug delivery vehicles, *Nanoscale*, **2013**, 5 (8), 3103.
4. Bhosale, S. V.; Kalyankar, M. B.; Nalage, S. V.; Lalander, C. H.; Bhosale, S. V.; Langford, S. J.; Oliver, R. F. pH dependent molecular self-assembly of octaphosphonate porphyrin of nanoscale dimensions: nanosphere and nanorod aggregates. *Int. J. Mol. Sci.*, **2011**, 12 (3), 1464.
5. Giacalone, G.; Hillaireau, H.; Capiau, P.; Chacun, H.; Reynaud, F.; Fattal, E. Stabilization and cellular delivery of chitosan–polyphosphate nanoparticles by incorporation of iron. *J. Control. Release*, **2014**, 194, 211.

www.agilent.com/chem

Printed in the USA, November 7, 2019
5994-1093EN

Agilent
Trusted Answers