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A Colon Targeted Delivery System for Resveratrol Enriching in pH Responsive-Model

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Introduction

According to the World Health Organization (WHO) report, colorectal cancer is the fourth most common form of cancer globally and the third leading cause of deaths among all cancers, accounting for 639,000 deaths worldwide per year.¹ Resveratrol is a promising candidate in prevention and treatment of colon cancer that has recently been well documented.²³ With respect to prevention or treatment of colon cancer by orally administered resveratrol, i.e. release of resveratrol in the colonic region at specific amount, maximal biological effect can be achieved.⁵ Designing colon-targeted resveratrol delivery system can increase resveratrol bioavailability at target site and reduce the administered dose and systemic side effects.⁶ Pectin has been used to formulate colon specific delivery because of resistance to the enzymes present in the stomach and intestine and complete degradation by the colonic bacterial enzymes.⁷⁸ Pectin can be administered to humans without any limits of daily intake.⁹ However, high swelling behavior of pectin and lack of reproducible performance of pectin formulations are two important challenges in the efficiency of pectin-based system and require more modification.¹⁰ Formation of polyelectrolyte complex between pectin as an anionic biopolymer

ABSTRACT

Background: Resveratrol effects on the prevention and treatment of colon cancer have been well documented recently, but low solubility, rapid absorption and metabolism of resveratrol limit its beneficial effects on colon cancer. Designing a formulation that enhances the solubility of resveratrol, protects resveratrol from oxidation and isomerization, and delivers it to the colon is a priority of food and drug industry. In this study, resveratrol-polyethylene glycol (PEG)-loaded pectin-chitosan polyelectrolyte complex was designed as a colon targeted delivery system.

Methods: The effects of adding PEG, ultra-sonication time, pH, and pectin to chitosan ratio were investigated on particle size, polydispersity index (PDI), zeta potential by particle size analyzer, and scanning electron microscopy (SEM). Encapsulation efficiency (EE), release of resveratrol in simulated gastrointestinal fluid, and different pHs were analyzed via High Performance Liquid Chromatography (HPLC). Antioxidant activity was measured by (2, 2-diphenyl-1-picryl-hydrazyl-hydrate) DPPH free-radical method.

Results: Results showed that colloidal stable micro-particles (725 ± 20 nm) with PDI < 0.3 and zeta potential +27 ± 2 mV was formed in the ratio of 5:1 of pectin to chitosan w/v % after a 10-min sonication. Encapsulation efficiency was 81 ± 7 %. The reduction of antioxidant activity of resveratrol loaded micro-particles after one month was less than 13%. Micro-particles released about 33% of resveratrol in the simulated gastric and intestinal fluids.

Conclusion: Two-thirds of the loaded resveratrol in Pectin-Chitosan complex reached colon. The developed system had enough specification for enriching fruit based drinks due to remarkable colloidal stability in the pH range of 3.5 to 4.5.
and chitosan as oppositely charged biopolymer enhances the water resistance of the resultant pectin complexes to some degree and overcomes the pectin high solubility in the small intestine.\textsuperscript{12,13} Chitosan has favorable biological properties, such as non-toxicity, biodegradability, tissue-adhesive activity, and anti-inflammatory response and offers excellent matrix for pH responsive drug release.\textsuperscript{14} Most of reported pectin-chitosan complexes for colon specific delivery were in the form of visible bead or capsule which were not suitable for food and beverages.\textsuperscript{15-20} The main concern of future food industry is enriching food and beverages with bioactive components in natural matrix for specific purpose.\textsuperscript{21} Therefore, development of micro- or nano-particles as colloidal suspension is a priority. The purpose of the present study was to characterize physicochemical properties, colloidal stability, and release behavior of resveratrol-polyethylene glycol(PEG)-loaded delivery system based on the complex of pectin-chitosan using particle size analyzer, zeta potential, HPLC, antioxidant activity, and SEM experiments. Thus, in this study, a resveratrol-loaded composite of pectin and chitosan was developed to design a food grade colon targeted colloidal delivery system.

**Materials and Methods**

**Materials**

Preparation of stock solution

Pectin stock solution (1.66 % w/v) was prepared by placing pectin in sterile bi-distilled water and agitated over night until complete hydration. Thereafter, it was centrifuged at 7000 rpm for 20 min and filtered with 0.45 µm syringe filter (cellulose acetate). The degree of esterification (DE) of pectin was determined according to Bocheket al. using titration method.\textsuperscript{22} Chitosan stock solution 1% w/v was prepared in 1% v/v acetic acid glacial and agitated over night until complete hydration, and then, it was diluted to lower concentration and centrifuged at 12000 rpm for 30 min and filtered with 0.22 µm syringe filter (cellulose acetate). The molecular weight was assayed by laser light scattering (Zetasizer ZS, Malvern instruments, UK). The pH was measured at room temperature (25°C) with pH meter (Metrohm, Germany). The pH of the pectin and chitosan solution was found to be \( \approx 3.6\pm0.1 \) and \( \approx 4.2\pm0.1 \), respectively.

Formulation of delivery system

Polyelectrolyte complexation was formed between two oppositely charge bio polymers at different concentration ratios.\textsuperscript{16,23} One milliliter of resveratrol solution in PEG (5 mM) was added to 9 mL pectin solution (1.66% w/v) under stirring at 500 rpm (Heidolph, Germany). After two hours stirring, the samples were sonicated for 0, 5, 10 and 15 min by probe sonicator (Hielscher, Germany) with a power of 200 W. Energy input was provided by a H3 sonotrode containing a piezoelectric crystal with a titanium probe of 3 mm in diameter. The amplitude of oscillation was set at 70 microns. All experiments were performed at room temperature, and the solution was stored in the refrigerator. The combination of pectin and resveratrol was added to chitosan solution in ratios of 1:1, 3:1, 5:1, and 10:1. It is worth noting that pectin concentration in all formulations was adjusted at 0.75 % w/v, and concentrations of the chitosan were 0.75%, 0.25, 0.15%, 0.075 % w/v.

**Antioxidant activity**

The potential antioxidant activity of delivery system was measured by the free radical scavenging effect on 2, 2- Diphenyl-1-picyrlhydrazyl (DPPH) radicals; this method is based on the reduction of DPPH in an alcoholic solution in the presence of a hydrogen-donating antioxidant.\textsuperscript{24} In its radical form, DPPH absorbs at 517 nm, but upon reduction by an antioxidant or a radical species, its absorption decreases.\textsuperscript{24,25} Radical scavenging activity of resveratrol solution in polyethylene glycol and pectin-chitosan complex in the presence and absence of resveratrol were measured according to Helal et al.\textsuperscript{26} In order to release resveratrol from delivery system and measure antioxidant activity, pectinase enzyme (0.6 mg/mL) was added to the samples.\textsuperscript{27} Then, the samples were shaken and incubated at 25 °C in a dark chamber for 30 min. The absorption of the samples was measured at 517 nm with a spectrophotometer (Pharmacia Biotech, England). A decrease in absorbance of the DPPH solution indicates increased DPPH radical scavenging activity. The values were calculated according to the following formula:\textsuperscript{28}

\[
\text{Scavenging effect (\%)} = \frac{\text{Blank absorbance} - \text{Sample absorbance}}{\text{Blank absorbance}} \times 100 \quad \text{Eq.(1)}
\]

**Scanning electron microscopy**

The morphology and structure of samples were visualized using scanning electron-microscope (SEM). To perform scanning electron microscopy, freeze dried samples was mounted on aluminum stubs. This was then gold coated in vacuum by a sputter. Samples were observed (KYKY-EM3200, Beijing, China) microscope. Photographs was taken at excitation voltage of 26 KV.

**Encapsulation efficiency**

Encapsulation efficiency (EE) of resveratrol in pectin-chitosan complex particles was assessed using Amicon centrifugal filter (Millipore, MW cut off 100,000Da).\textsuperscript{28} The resveratrol loaded particles were placed on the upper compartment of Amicon® tubes and centrifuged at 4000 rpm for 30
min to separate free resveratrol from the lower compartment of Amicon according to Pandita et al. with some modifications.\textsuperscript{29} A volume of 500 µL of filtrated solution was removed and mixed with 500 µl ethanol. A volume of 50 µL of mixture was injected into HPLC-UV:\textsuperscript{30}

\[
\text{EE (\%)} = \frac{\text{Total resveratrol} - \text{Free resveratrol}}{\text{Total resveratrol}} \times 100 \quad \text{Eq.(2)}
\]

Release behavior at different pH values
Release behavior was assayed in four different pH values by incubating 1 mL of selected formulation inside the dialysis bag suspended in 20 mL of acetate buffer. The pH was adjusted at pH 2.5, pH 3.5, pH 4.5 and pH 5.5 by 0.1 M acetic acid in one month at 4°C. Released resveratrol from delivery system was measured in sequence7 days and the same volume of fresh incubation medium was replaced after every sampling.

Release behavior at simulated gastrointestinal fluid
Cumulative release of resveratrol from micro-particles was evaluated by dialysis bag diffusion technique (cut off value =11000 Da) by mimicking oral delivery to colon specific site. The particles’ transit condition was set as gastric condition (pH 1.2 for 2 h for stomach), intestinal condition (pH 6.8 for 3 h small intestine), and colon (pH 7.4 for 2 h) in presence and absence of pectinase.\textsuperscript{31} To mimic stomach condition without enzymes under sink condition, 1mL of sample containing resveratrol was added into the dialysis bag and suspended in 20 mL of HCl (0.1 M)-ethanol w/v (80:20 ratio). Thereafter, dialysis bag was suspended in 20 mL phosphate buffer-ethanol (ratio 80:20) to mimic small intestine condition. For simulated colon condition, the buffer was changed (pH 7.4) in the presence and absence of 0.6 mg/mL pectinase enzyme under stirrer in an incubator (50 rpm, 37°C) (shaker incubator, Heidolph unimax 1010, Germany). Resveratrol content was assessed at the end of simulation.

Statistical analysis
All the experiments were performed three times independently with triplicates and the results were expressed as mean ± standard deviation. Statistical analysis was carried out using Graph Pad Prism software to find the statistical significance of these values. A probability of \( p < 0.05 \) was considered to be statistically significant.

Results
Pectin-chitosan (P/C) complex
The degree of esterification (DE) of pectin was determined 70 ± 2 % w/v, and the average molecular weight was around 120 kDa. To increase resveratrol solubility, PEG (4 kDa) solution was used. Resveratrol was easily dissolved in PEG to concentration of 1.64 M in preliminary experiments. As shown in Figure 1, adding PEG to pectin solution and the ultra-sonication for 10 min had a significant effect on particle size reduction.

PEG increased viscosity of media and cavitation intensity during ultra-sonication.\textsuperscript{32} Zeta potential of ultra-sonicated pectin solution increased from -15 mV to about -30 mV. Particle size decreased from 1220 to 760 nm. The PDI was in acceptable range after 10 min sonicron (PDI<0.3). Then, this formula was selected to form the polyelectrolyte complex with chitosan. According to Figure 2, by increasing the concentrations of chitosan from 0.075 to 0.75%w/v, at constant pectin concentration (0.75% w/v), the zeta potential increased from -3 ± 0.7 mV (P/C, 10:1 %w/v) to +43 ± 1.8 mV (P/C, 1:1 %w/v), and consequently, the particle size decreased from 861 ± 42 to 622 ± 21 nm. The pH of samples was set at 4.5±0.1.
Micro-particles formed on 1:1, 3:1, and 5:1 P/C ratios were stable but complex on 10:1 P/C ratio was unstable during storage time, which may be related to lower electrostatic repulsion. Homogenous particles with appropriate PDI were achieved at 5:1 P/C (particle size 725 ± 20 nm and zeta potential +27 ± 2 mV, pH=4.5 ± 0.1). Therefore, this formulation was selected for resveratrol loading due to fewer changes in their size and PDI during 30 days storage at 4°C. Figure 3 (A) shows the SEM image and morphology of micro-particles that verify the measured size using DLS.

**Encapsulation efficiency**

As shown in Figure 4, EE of pectin: chitosan micro-particles at 10:1 were significantly lower than other formulations. The EE of micro-particles on 5:1, 3:1, and 1:1 ratios was more than 80% and wasn’t significantly different. Thus, due to homogenous size distribution of complex in the ratio of 5:1, it was chosen as an appropriate formulation for encapsulation of resveratrol.

![Figure 2.](image2.png)

Figure 2. Effect of different pectin: chitosan ratio on particle size (A), PDI (B), Zeta potential (C) in first day and after thirty days.

![Figure 3.](image3.png)

Figure 3. SEM image (A), Particle size (B), Zeta potential of resveratrol- PEG loaded pectin-chitosan particles (pectin: chitosan, 5:1).
Colon, Delivery, Resveratrol

Table 1. Cumulative release of resveratrol at different pH values during one month (%).

| Day  | 2.5    | 3.5    | 4.5    | 5.5    |
|------|--------|--------|--------|--------|
| 2<sup>nd</sup> | 0.33±0.03<sup>a</sup> | 0.29±0.02<sup>a</sup> | 0.14±0.03<sup>b</sup> | 0.51±0.03<sup>c</sup> |
| 8<sup>th</sup> | 7.37±0.49<sup>a</sup> | 5.63±0.37<sup>b</sup> | 4.24±0.25<sup>c</sup> | 14.20±1.21<sup>d</sup> |
| 15<sup>th</sup> | 11.47±1.48<sup>a</sup> | 9.56±0.60<sup>b</sup> | 7.35±0.70<sup>c</sup> | 20.41±2.32<sup>d</sup> |
| 22<sup>nd</sup> | 17.06±1.53<sup>a</sup> | 12.42±0.93<sup>b</sup> | 9.48±0.91<sup>c</sup> | 26.34±2.94<sup>d</sup> |
| 29<sup>th</sup> | 21.04±2.02<sup>a</sup> | 18.91±1.41<sup>b</sup> | 13.74±1.32<sup>c</sup> | 44.75±3.53<sup>d</sup> |

Different words indicate significant difference at the 5% level in Duncan’s test.

Figure 4. Encapsulation efficiency of different formulations after preparation.

Release behavior at different pH values
In order to evaluate the stability of the system at different pH values (2.5, 3.5, 4.5, and 5.5), the release behavior of resveratrol was assessed by HPLC during one month with seven days interval at 4 °C (Table 1). Obviously, no initial burst release of resveratrol was observed in different pH values. The resveratrol released in pH 4.5 was lower than other pHs during storage (13.74 ± 1.32%), and the maximum amount of release was determined at pH 5.5 with 44.75 ± 3.53% at the end of the one-month period.

Release behavior in simulated gastrointestinal fluid
The amount of released resveratrol in simulated gastric and small intestinal condition was 5 ± 0.5% (pH=1.2 in absence of enzymes) and 16 ± 0.8% (pH=6.8), respectively. The cumulative release of resveratrol before reaching the large intestine was around 21%. Furthermore, at pH=7.4 and in absence of pectinase, the resveratrol release was 22 ± 1.8%. Indeed, cumulative release of resveratrol in absence of pectinase was about 43%, while in presence of pectinase at pH=7.4, the entire resveratrol was released.

Antioxidant activity
Figure 5 shows that the antioxidant activity of pectin-chitosan complex without resveratrol was 37 ± 5%, which is mainly attributed to the presence of chitosan. Antioxidant activity of encapsulated resveratrol was 93 ± 6% in comparison to the pure form of resveratrol (72 ± 3.5%). Antioxidant activities of free resveratrol and encapsulated form were measured after 30 days storage at 4º C. The results indicated that the antioxidant activity of encapsulated form decreased 13 ± 2% whereas the reduction of free resveratrol was 48 ± 4%.

Discussion
Pectin-chitosan (P/C) complex
Polyelectrolyte complex of pectin and chitosan was stabilized via attractive force between carboxyl groups of pectin and amine groups of chitosan. The pKa value of chitosan was 6.3; hence, in the 2.0–5.0 pH range, most of chitosan amine groups were protonated. In contrast, the degree of ionization of pectin decreased with decreasing pH values (pKa= 3.6). Therefore the presence of optimum mole of repeating units of pectin and chitosan is a critical control point in formulation of a colloidal stable suspension with compact structure; for example, much larger amounts of pectin were required to interact with chitosan at low pH values. Based on the results, stable
colloidal suspension with appropriate PDI was achieved in the P/C ratio of 5:1 (particle size 725 ± 20 nm and zeta potential +27 ±2 mV, pH=4.5 ± 0.1). It was shown in previous studies that polymer concentration, addition order, mass ratio, and pH solution had important influences on the formation of polyelectrolyte complex and particles characteristics.35-37 High electrostatic repulsion between particles suggests a structure whose surface is dominated by chitosan.37

Encapsulation efficiency
The low solubility and loading capacity are two critical limitations in designing delivery system for resveratrol.15 Thus, the current formulated micro-particles could adjust the loaded resveratrol for achieving the defined amount of resveratrol for colon because of high solubility of resveratrol in PEG. It was reported that the EE of progesterone loaded pectin-zinc-chitosan in the concentrations of 1, 4, 4, and 1 % w/v was around 75 % with the size of 500-700 µm.30 EE of albumin in pectin-chitosan complex was around 58-73%.38

Release behavior at different pH values
Low water solubility of resveratrol induced to cumulative release in different pH values (2.5, 3.5, and 4.5) had no significant difference during 30 days storage. The pH of pectin-chitosan complex influences the charge density of system and release behavior of resveratrol.15 The release of resveratrol from micro-particles at pH 4.5 was lower than other pH values during 30 days (13.7 ±1.3%). It would be related to the fact that pectin is fully charged at pHs above 3.5. Therefore, at pHs 4-5, stronger complex is formed, also the highest release at pH 5.5 is probably related to this fact that as the pH increases toward 6.0, amine group of chitosan is deprotonated; therefore, some complex is decomposed.23

Release behavior in simulated gastrointestinal fluid
The results of presence study are almost in agreement with recent studies and the release of encapsulant in upper pHs is more than lower pHs. Das et al.reported that pectin-zinc-chitosan micro-particles released approximately 4.5 %, 40 %, and 95 % of loaded resveratrol during transition from simulated gastric, intestinal, and colonic fluids, respectively.14 In another study, < 25 % of loaded resveratrol in zinc pectinate beads was released in pH 1.5 after 2 hours, and almost 50% of the loaded resveratrol was released in pH 6.3 after 3 hours.15 In present study in presence of pectinate at pH=7.4, the entire resveratrol was released, indicating that once the complex reaches the colon, it is decomposed by microbial pectinase, and the entire encapsulant is released from the complex structure.

Antioxidant activity
In presence of chitosan, antioxidant activity of encapsulated resveratrol was significantly more than pure form of resveratrol, indicating synergetic antioxidant effects of resveratrol with chitosan.39 In addition, the reduction of antioxidant activity of encapsulated resveratrol was significantly lower than free resveratrol after one month. This is in line with previous studies in which encapsulation prevented the reduction of antioxidant activity by time.25

Conclusion
This study was conducted to develop a delivery system based on pectin and chitosan containing resveratrol. PEG enhanced the solubility of resveratrol and reduced the average particle size. Ultra-sonication of pectin enhanced zeta potential. The resultant micro-particles of pectin-chitosan had smaller particles with suitable PDI at 5:1 (pH=4.5). Release behavior of resveratrol in simulated gastrointestinal fluids showed that more than two thirds of the loaded resveratrol reached colon, more than the minimum amounts (50 µM) of resveratrol for showing its therapeutic effects. It seems that the developed system has enough specification for enriching fruit based drinks due to remarkable colloidal stability in the pH range of 3.5 to 4.5.

Conflict of interests
The authors claim that there is no conflict of interest.

Reference
1. Perfly J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: Globocan 2008. Int J Cancer. 2010;127(12):2893-917. doi:10.1002/jic.25516
2. Cui X, Jin Y, Hofseth AB, Pena E, Habiger J, Chumanevich A, et al. Resveratrol suppresses colitis and colon cancer associated with colitis. Cancer Prev Res. 2010;3(4):549-59. doi:10.1158/1940-6207.CAPR-09-0117
3. Bishayee A. Cancer prevention and treatment with resveratrol: From rodent studies to clinical trials. Cancer Prev Res. 2009;2(5):409-18. doi:10.1158/1940-6207.CAPR-08-0160
4. Aggarwal BB, Bhardwaj A, Aggarwal RS, Seeram NP, Shishodia S, Takada Y. Role of resveratrol in prevention and therapy of cancer: Preclinical and clinical studies. Anticancer Res. 2004;24(5A):2783-840.
5. Juan ME, Alfaras I, Planas JM. Colorectal cancer chemoprevention by trans- resveratrol. Pharmacol Res. 2012;65(6):584-91. doi:10.1016/j.phrs.2012.03.010
6. Augustin MA, Sanguansri L, Lockett T.
Nano-and micro-encapsulated systems for enhancing the delivery of resveratrol. Ann N Y Acad Sci. 2013;1290(1):107-12. doi:10.1111/nyas.12130

7. Shukla RK, Tiwari A. Carbohydrate polymers: Applications and recent advances in delivering drugs to the colon. Carbohydr Polym 2012;88(2):399-416. doi:10.1016/j.carbpol.2011.12.021

8. Auriemma G, Mencherini T, Russo P, Stigliani M, Aquino RP, Del Gaudio P. Prilling for the development of multi-particulate colon drug delivery systems: Pectin vs. Pectin–alginate beads. Carbohydr Polym. 2013;92(1):367-73. doi:10.1016/j.carbpol.2012.09.056

9. Liu L, Fishman ML, Kost J, Hicks KB. Pectin-based systems for colon-specific drug delivery via oral route. Biomaterials. 2003;24(19):3333-43. doi:10.1016/s0142-9612(03)00213-8

10. Ashford M, Fell J, Attwood D, Sharma H, Woodhead P. An evaluation of pectin as a carrier for drug targeting to the colon. J Control Release. 1993;26(3):213-20. doi:10.1016/0168-3659(93)90188-b

11. Kosaraju SL. Colon targeted delivery systems: Review of polysaccharides for encapsulation and delivery. Crit Rev Food Sci Nutr. 2005;45(4):251-8. doi:10.1080/10408690490478091

12. Ribeiro LN, Alcântara AC, Darder M, Aranda P, Araújo-Moreira FM, Ruiz-Hitzky E. Pectin-coated chitosan–ldh bionanocomposite beads as potential systems for colon-targeted drug delivery. Int J Pharm. 2014;463(1):1-9. doi:10.1016/j.ijpharm.2013.12.035

13. Luo Y, Wang Q. Recent development of chitosan-based polyelectrolyte complexes with natural polysaccharides for drug delivery. Int J Biol Macromolec. 2014;64:353-67. doi:10.1016/j.ijbiomac.2013.12.017

14. Gulbake A, Jain SK. Chitosan: A potential polymer for colon-specific drug delivery system. Expert Opin Drug Deliv. 2012;9(6):713-29. doi:10.1517/17425247.2012.682148

15. Das S, Chaudhury A, Ng K-Y. Preparation and evaluation of zinc–pectin–chitosan composite particles for drug delivery to the colon: Role of chitosan in modifying in vitro and in vivo drug release. Int J Pharm. 2011;406(1-2):11-20. doi:10.1016/j.ijpharm.2010.12.015

16. Das S, Ng K-Y. Colon-specific delivery of resveratrol: Optimization of multi-particulate calcium-pectinate carrier. Int J Pharm. 2010;385(1):20-8. doi:10.1016/j.ijpharm.2009.10.016

17. Puga AM, Lima AC, Mano JF, Concheiro A, Alvarez-Lorenzo C. Pectin-coated chitosan microgels crosslinked on superhydrophobic surfaces for 5-fluorouracil encapsulation. Carbohydr Polym. 2013;98(1):331-40. doi:10.1016/j.carbpol.2013.05.091

18. Bigucci F, Luppi B, Cerchiara T, Sorrenti M, Bettinetti G, Rodriguez L, et al. Chitosan/pectin polyelectrolyte complexes: Selection of suitable preparative conditions for colon-specific delivery of vancomycin. Eur J Pharm Sci. 2008;35(5):435-41. doi:10.1016/j.ejps.2008.09.004

19. Yao KD, Tu H, Cheng F, Zhang JW, Liu J. Ph-sensitivity of the swelling of a chitosan-pectin polyelectrolyte complex. Angewandte Makromolekulare Chemie. 1997;245(1):63-72. doi:10.1002/apmc.1997.052450106

20. Gadalla HH, Soliman GM, Mohammed FA, El-Sayed AM. Development and in vitro/in vivo evaluation of zn-pectinate microparticles reinforced with chitosan for the colonic delivery of progesterone. Drug Deliv. 2015;23(7):2541-54. doi:10.3109/10717544.2015.1028602

21. Benshiriti RC, Levi CS, Tal SL, Shimonri E, Lesmes U. Development of oral food-grade delivery systems: Current knowledge and future challenges. Food Funct. 2012;3(1):10-21. doi:10.1039/c1fo10068h

22. Bochek A, Zabivalova N, Petropavlovskii G. Determination of the esterification degree of polygalacturonic acid. Russ J Appl Chem. 2001;74(5):796-9. doi:10.1023/A:1012701219447

23. Birch NP, Schiffman JD. Characterization of self-assembled polyelectrolyte complex nanoparticles formed from chitosan and pectin. Langmuir. 2014;30(12):3441-7. doi:10.1021/la500491c

24. Bondet V, Brand-Williams W, Berer C. Kinetics and mechanisms of antioxidant activity using the dpph. Free radical method. LWT-Food Sci Technol. 1997;30(6):609-15. doi:10.1006/lwt.1997.0240

25. Pastor C, Sánchez-Gonzállez L, Chiralt A, Cháfer M, González-Martínez C. Physical and antioxidant properties of chitosan and methylcellulose based films containing resveratrol. Food Hydrocoll. 2013;30(1):272-80. doi:10.1016/j.foodhyd.2012.05.026

26. Helal A, Tagliazucchi D, Conte A, Desobry S. Antioxidant properties of polyphenols incorporated in casein/sodium caseinate films. Int J Food Sci Technol. 2013;48(1):10-5. doi:10.1111/ijfs.12148

27. Yu C-Y, Yin B-C, Zhang W, Cheng S-X, Zhang X-Z, Zhuo R-X. Composite microparticle drug delivery systems based on chitosan, alginate and pectin with improved ph-sensitive drug release property. Colloids Surf B Biointerfaces.
28. Gomes LP, Souza HK, Campiña JM, Andrade CT, Paschoalin VMF, Silva AF, et al. Tweaking the mechanical and structural properties of colloidal chitosans by sonication. Food Hydrocoll. 2016;56:29-40. doi:10.1016/j.foodhyd.2015.11.021

29. Pandita D, Kumar S, Poonia N, Lather V. Solid lipid nanoparticles enhance oral bioavailability of resveratrol, a natural polyphenol. Food Res Int. 2014;62:1165-74. doi:10.1016/j.foodres.2014.05.059

30. Sanna V, Roggio AM, Siliani S, Piccinini M, Marceddu S, Mariani A, et al. Development of novel cationic chitosan- and anionic alginate-coated poly (d, l-lactide-co-glycolide) nanoparticles for controlled release and light protection of resveratrol. Int J Nanomedicine. 2012;7:5501-16. doi:10.2147/IJN.S36684

31. Krivorotova T, Cirkovas A, Maciulyte S, Staneviciene R, Budriene S, Serviene E, et al. Nisin-loaded pectin nanoparticles for food preservation. Food Hydrocoll. 2016;54:49-56. doi:10.1016/j.foodhyd.2015.09.015

32. Behrend O, Ax K, Schubert H. Influence of continuous phase viscosity on emulsification by ultrasound. Ultrason Sonochem. 2000;7(2):77-85. doi:10.1016/s1350-4177(99)00029-2

33. Coimbra P, Ferreira P, De Sousa H, Batista P, Rodrigues M, Correia I, et al. Preparation and chemical and biological characterization of a pectin/chitosan polyelectrolyte complex scaffold for possible bone tissue engineering applications. Int J Biol Macromol. 2011;48(1):112-8. doi:10.1016/j.ijbiomac.2010.10.006

34. Sæther HV, Holme HK, Maurstad G, Smidsrød O, Stokke BT. Polyelectrolyte complex formation using alginate and chitosan. Carbohydr Polym. 2008;74(4):813-21. doi:10.1016/j.carbpol.2008.04.048

35. Schatz C, Lucas J-M, Viton C, Domard A, Pichot C, Delair T. Formation and properties of positively charged colloids based on polyelectrolyte complexes of biopolymers. Langmuir. 2004;20(18):7766-78. doi:10.1021/la049460m

36. Schatz C, Domard A, Viton C, Pichot C, Delair T. Versatile and efficient formation of colloids of biopolymer-based polyelectrolyte complexes. Biomacromolecules. 2004;5(5):1882-92. doi:10.1021/bm049786+

37. Andishmand H, Tabibiazar M, Mohammadi Far MA, Hamishehkar H. Pectin-zinc-chitosan-polyethylene glycol colloidal nano-suspension as a food grade carrier for colon targeted delivery of resveratrol. Int J Biol Macromol. 2017;97:16-22. doi:10.1016/j.ijbiomac.2016.12.087

38. Kim TH, Park YH, Kim KJ, Cho CS. Release of albumin from chitosan-coated pectin beads in vitro. Int J Pharm. 2003;250(2):371-83. doi:10.1016/s0378-5173(02)00553-7

39. Perchyonoka VT, Zhangb S, Oberholzer T. Protective effect of conventional antioxidant (-carotene, resveratrol and vitamin e) in chitosan-containing hydrogels against oxidative stress and reversal of DNA double stranded breaks induced by common dental composites: In-vitro model. The Open Nanoscience Journal. 2013;7(1):1-7. doi:10.2174/1874140101307010001