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Design and Development of the Chitosan-Rosuvastatin complex for the Enhancement of Bioavailability and Safety of Rosuvastatin.

Mohd. Imran[1]*, Faiyaz Shakeel[2], Abdulhakim Bawadekji[3], and Mouhanad Al Ali[4].

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Northern Border University, P.O. Box 840, Rafha 91911, Saudi Arabia.
[2]Department of Pharmaceutics, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia.
[3]Deanship of Scientific Research, Northern Border University, P.O. Box 1321, Arar 91431, Saudi Arabia.
[4]Université d'Angers, Institut Supérieur de la Santé et des Bioproduits d'Angers, Angers 49045, France.

ABSTRACT

The objective of this research work was to develop chitosan based polymeric complex of rosuvastatin for improving its solubility and stability. The chitosan-rosuvastatin complex was prepared by using amide coupling reaction. The coupling reaction was carried out by reaction between the amine groups of the chitosan and the activated carboxylic group of the rosuvastatin. The prepared complex was characterized by its ^{1}H-NMR, FTIR, X-ray diffraction, and zeta potential. The chitosan-rosuvastatin complex was subjected for its muco-adhesive property, solubility studies, acid degradation studies, and the radical scavenging activity. The results suggested that the chitosan-rosuvastatin complex retains the muco-adhesiveness of the parent chitosan; its solubility was approximately 250-fold higher than that of pure rosuvastatin; it demonstrated nearly 3-fold higher stability than the rosuvastatin; and it exhibited higher DPPH radical scavenging activity as compared to the rosuvastatin. Based on the results, it has been concluded that the chitosan-rosuvastatin complex has a potential to demonstrate enhanced bioavailability *in vivo*. However, further *in vivo* studies are recommended.

Keywords: Rosuvastatin, Chitosan, Complex, Solubility, Stability, Bioavailability.

Corresponding author

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INTRODUCTION

Rosuvastatin (RS) is a lipid regulating drug, which competitively inhibits hydroxy-methylglutarylcoenzyme A (HMG-CoA) reductase and inhibits cholesterol synthesis cholesterol [1-3]. Among the several statins available for clinical management of hyperlipidemia, RS is considered to be well tolerated in the dose of 10-80 mg to reduce the total blood cholesterol and low-density lipids (LDL). It also modifies apolipoprotein and lipid in patients with hypercholesterolaemia, mixed dyslipidaemia, and hypertriglyceridaemia and is used to reduce the progression of atherosclerosis [4, 5]. Several clinical trials have reported superior efficacy of RS over other statins [6].

However, RS belongs to BCS class II with crystalline nature and low aqueous solubility of around 0.089 mg/ml. Also, like other statins, RS is unstable under conditions of acidity, oxygen and light and forms lactone degradation product. Both sparingly soluble nature and instability under physiological conditions of the stomach result in its low oral bioavailability, which was approximately 20% [7, 8]. Enhancing the stability and solubility are thus the desirable approaches to improve its therapeutic performance. Several delivery systems have been explored for augmenting the oral bioavailability of RS primarily by augmenting the solubility, yet the results indicate limited success of the formulations developed so far. Development of polymer complex is considered as a novel tool for product development. The polymeric complex approach has attracted considerable attention due to their particular therapeutic properties, such as prolonged half-life, solubility enhancement, stabilization and bioavailability enhancement [9, 10]. Hence, it could serve as an effective tool to overcome the drawbacks associated with oral delivery of RS. Thus, in the present studies, we have explored the possibility of developing polymeric complexes of RS for improving its biopharmaceutical properties. Chitosan (CH), a cationic polysaccharide, was selected for the complexation purpose in the current project because of its biocompatibility, biodegradability, non-irritability upon oral administration [11, 12]. Thus, the aim of current research was to develop chitosan based polymeric complex of RS for improving its solubility and stability.

MATERIALS AND METHODS

Materials

RS was obtained as a gift sample from Ranbaxy Laboratories Ltd. (Himachal Pradesh, India). Chitosan (CH) (ChitoClear™, degree of acetylation 96%) was obtained from Primex ehf (Siglufjordur, Iceland). 1-Ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC) was purchased from Himedia Laboratories (Mumbai, India). All other chemicals were of analytical grade and were used as received from Merck Ltd. (Mumbai, India).

Synthesis of CH–RS complex

CH–RS complex was prepared by using amide coupling reaction [13]. A RS solution 5% (w/v) in chloroform (10 mL) was activated by EDC (125 mM, 2 mL) treatment for 6 h at room temperature to afford an ester form of RS. Separately, 1% (w/v) aqueous CH solution was prepared after hydrating CH with 1 N HCl (5 mL). The methanolic solution of RS was then added dropwise to the aqueous acidic CH solution under continuous magnetic stirring. Throughout the experiment, pH was maintained in the range of 5–6. After stirring for 24 h at room temperature, the excess reagent and the corresponding acylisourea (by-product after coupling) was removed by washing with distilled water. The reaction mixture was then purified using ultrafiltration, after which the CH–RS complex was lyophilized. Quantification was done using UV spectroscopy at absorption maxima 252 nm.

Characterization of CH-RS complex

^{1}H-Nuclear magnetic resonance (NMR) spectroscopy

^{1}H-NMR spectra of CH, RS and CH–RS complex in D_2O were recorded at 300 MHz FT-NMR (Bruker DRX-300, US) with low (−90°C) and high (+80°C) temperature facility, using TMS as the internal standard. Chemical shift (δ) values were given in parts per million (ppm).

FT-IR analysis

The chemical structure of the CH–RS complex was characterized by using FT-IR spectroscopy in order to identify the linkage generated between CH and RS. Briefly, CH, RS and the CH-RS complex were separately mixed with KBr (1:1) and converted into a pellet and were scanned between 4000 to 500 wavelength ranges (cm^{-1}).

Zeta potential

Measurement of the zeta potential of complex was done using the Zetasizer (Nano ZS, Malvern Instruments, Malvern, UK). For zeta potential measurements, disposable capillary cell with a capacity of 1 mL was used. To obtain a complete dispersion, the complexes were dispersed in Marcol 52 (Exxon Mobil Co., USA) and sonicated for 10 min at 120 W power (Branson 8210, Branson Ultrasonics Co., Danbury, CT, USA).

X-ray diffraction

The physical form of the lyophilized complex was determined by powder X-ray diffraction over a range of 2h from 5° to 60° with Ni-filered Cu-Ka radiation. The scan speed was 3 min^{-1}.

Muco-adhesiveness of CH-RS complex

The muco-adhesive property of the suspension of the RS and CH–RS complex was evaluated by an *in vitro* adhesion testing method known as the wash-off method. Freshly-excised pieces of intestinal mucosa of rat were mounted onto glass slides with cyanoacrylate glue. Two glass slides were connected with a suitable support. About 50 µL of each sample was spread onto each wet rinsed tissue specimen, and immediately thereafter, the support was hung onto the arm of a USP tablet disintegrating test machine. When the disintegrating test machine was operated, the tissue specimen was given a slow, regular up-and-down movement in the test fluid (400 mL) at 37°C contained in a 1000 mL vessel of the machine. At the end of 4 h, the machine was stopped and the remaining amount of drug adhering to the tissue was quantified by the spectroscopy.

Solubility of CH-RS complex

Solubility was evaluated by adding an excess of RS and CH–RS complex to the deionized water (10 mL) in screw-capped tubes placed in a water-jacketed vessel linked to a temperature-controlled water bath maintained at 37 ± 0.1 °C for 48 h. Continuous agitation was provided by overhead stirring. Each sample was centrifuged at 18,000 rpm for 30 min and the respective clear supernatants were diluted with methanol and analyzed by UV spectroscopy.

Acidic degradation studies of CH-RS complex

Stability of RS and CH-RS complex in conditions simulating the gastric environment was determined by adding 10 µg of RS and CH–RS complex to 10 mL of 1 N HCl and the mixture was refluxed at 80°C. At designated time points, 5 mL of the sample was withdrawn and assayed for RS concentration by UV spectroscopy.

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Radical-scavenging activity (RA)

DPPH radical-scavenging activity was performed by the reported method [14]. Suspension of RS and CH-RS complex was prepared at a concentration of 1 mg/mL and was mixed with a methanolic solution of DPPH (5 mL, 0.06 mM). The mixtures were shaken vigorously and incubated at 37°C in the dark for 30 min. At the same time, a control containing water (1.0 mL) and methanolic solution of DPPH (5 mL, 0.06 mM) was run. The absorbance was measured at 517 nm against methanol as a blank. The percentage of DPPH scavenging was calculated as follows:

$$RA\ (\%) = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

All determinations were carried out in triplicate. Ascorbic acid was used as a reference compound.

RESULTS AND DISCUSSION

Synthesis of CH–RS complex

RS is a poor water soluble, low bioavailability compound, belonging to BCS class II. It is also known that HMG-CoA reductase inhibitors including rosuvastatin are unstable under conditions of acidity (GI conditions) and oxidation, and form lactone resulting in reduced activity and bioavailability. On the other hand CH is a hydrophilic water soluble macromolecule. It is muco-adhesive in nature and is also known to improve permeation of drug molecules across biological barriers. In the present work intent is to react amine-functional groups of CH with carboxylic groups of rosuvastatin to form complex that could probably be more soluble, stable, effective and bioavailable. The chemical structure of CH–RS complex is shown in Fig. 1.

RS was covalently attached to CH through an amide linker that is known to be cleaved under physiological conditions. The complexation between CH and RS was carried out using amide coupling reaction between the amine groups of CH and activated carboxylic group of RS (Fig. 1). The carboxylic group of RS was activated using EDC by the formation of O-acylisourea, which could be viewed as a carboxylic ester with an activated leaving group (Fig. 1). EDC was selected because of its solubility in a wide range of solvents and easy separation of its by-product.

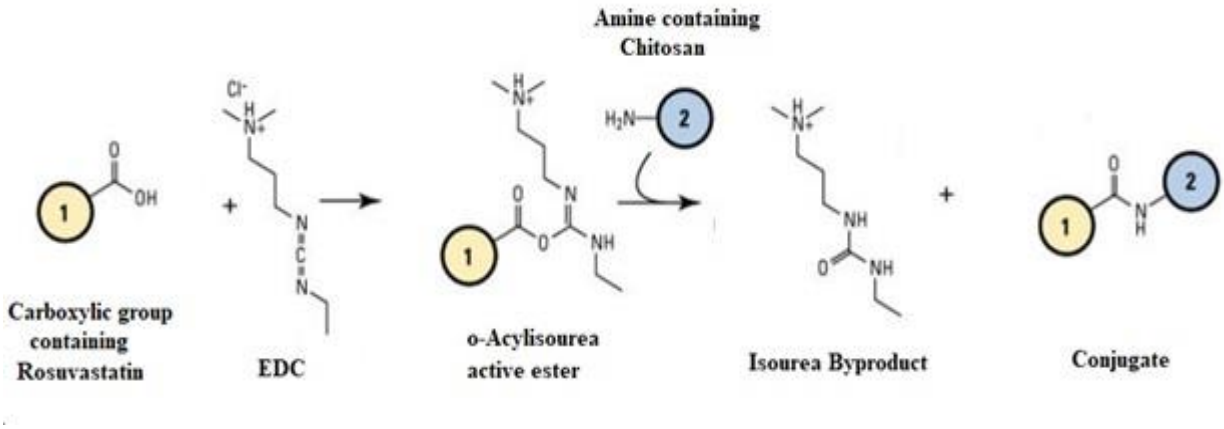

Fig. 1: Schematic representation of the CH-RS complex formation

Characterization of CH-RS complex

The complex was characterized by ^{1}H-NMR, showing peaks corresponding to both CH and RS, and a distinctive peak at δ value of 9.5 owing to amide bond formation (Fig. 2C).

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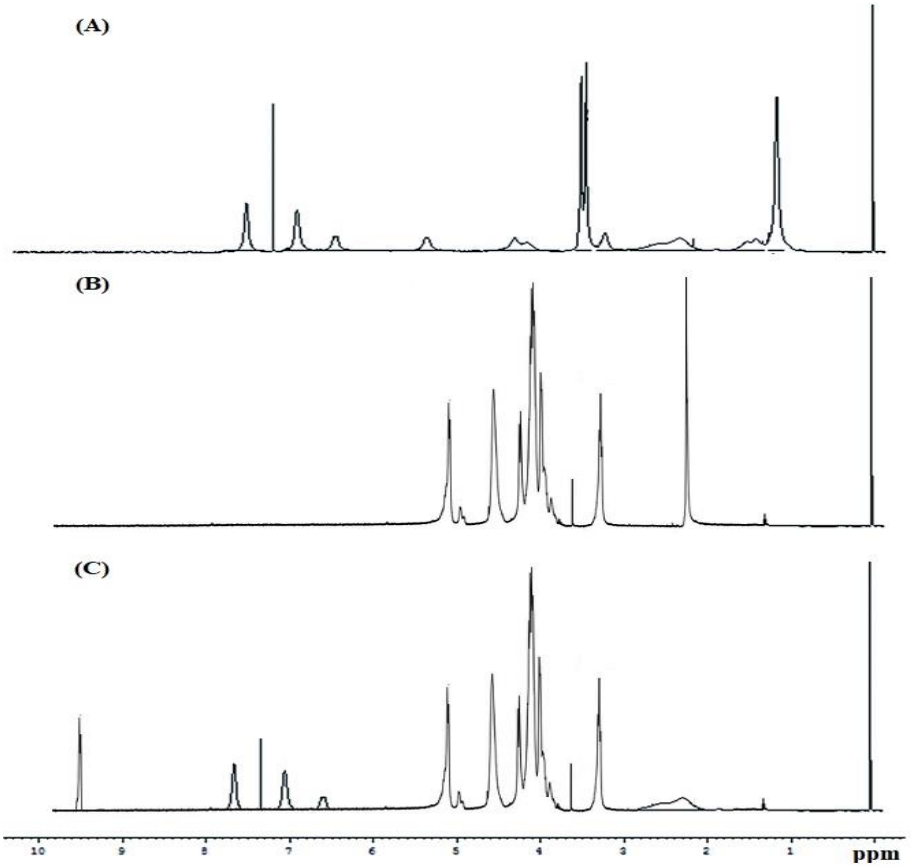

Fig. 2: ^{1}H-NMR spectrum of RS (A), CH (B), CH–RS complex (C)

The same has been confirmed by a distinctive peak at 1700 cm^{-1} in FT-IR spectrum of CH–RS complex (Fig. 3C). Further, the absence of unsaturated carbon–carbon double bond peaks at 1420 cm^{-1} (Fig. 3B) and displacement of the secondary amine deformation band from 1550 (Fig. 3A) to 1480 cm^{-1} (Fig. 3C), suggests that the coupling reaction had occurred between the amino group of chitosan and the carboxylic group of RS. The weight percentage (% w/w) of RS in the CH–RS complex as quantified using the spectroscopy was found to be 28% (w/w).

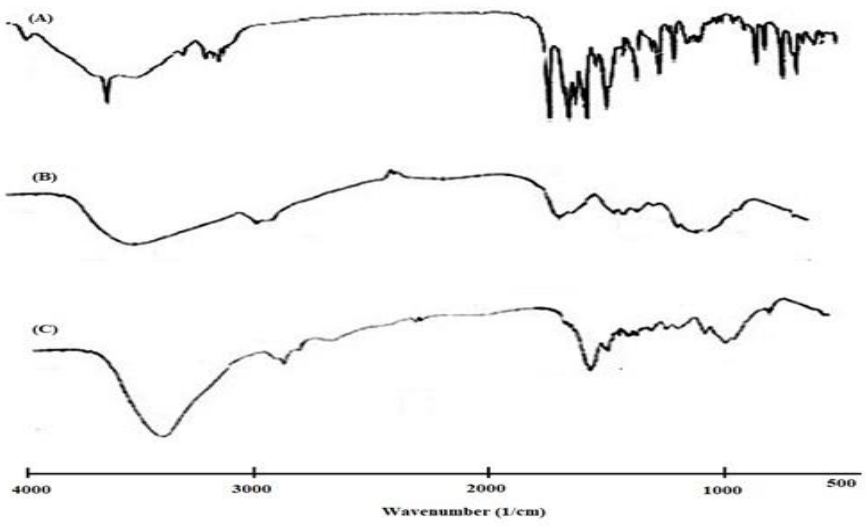

Fig. 3: FTIR spectra of RS (A), chitosan (B), CH–RS complex (C)

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Results of zeta potential shows change in value of -11.8 mV for RS to + 13.5 mV for CH-RS complex. The results indicate that complexation of CH (zeta potential, +26.2 mV) resulted in charge suppression of RS. The positive surface charge could be further useful for providing muco-adhesive property to the complex.

In order to identify the physical state and crystallinity of RS in polymeric complex, the XRD spectra of pure RS, CH, and CH–RS complex are presented in Fig. 4. As can be seen from the Fig. 5, pure RS showed sharp peaks at 2θ scattered angles of 14.2, 22.6, 34.5, 45.4 and 65.2° an indication of the crystalline nature of the drug. The CH powder showed two major broad crystalline peaks at 2h of around 9.5° and 19.7°, respectively, while the diffraction peaks of the CH–RS complex showed the amorphous nature of the complex. The possibility of amorphous drug formation due to attachment of CH to RS cannot be neglected.

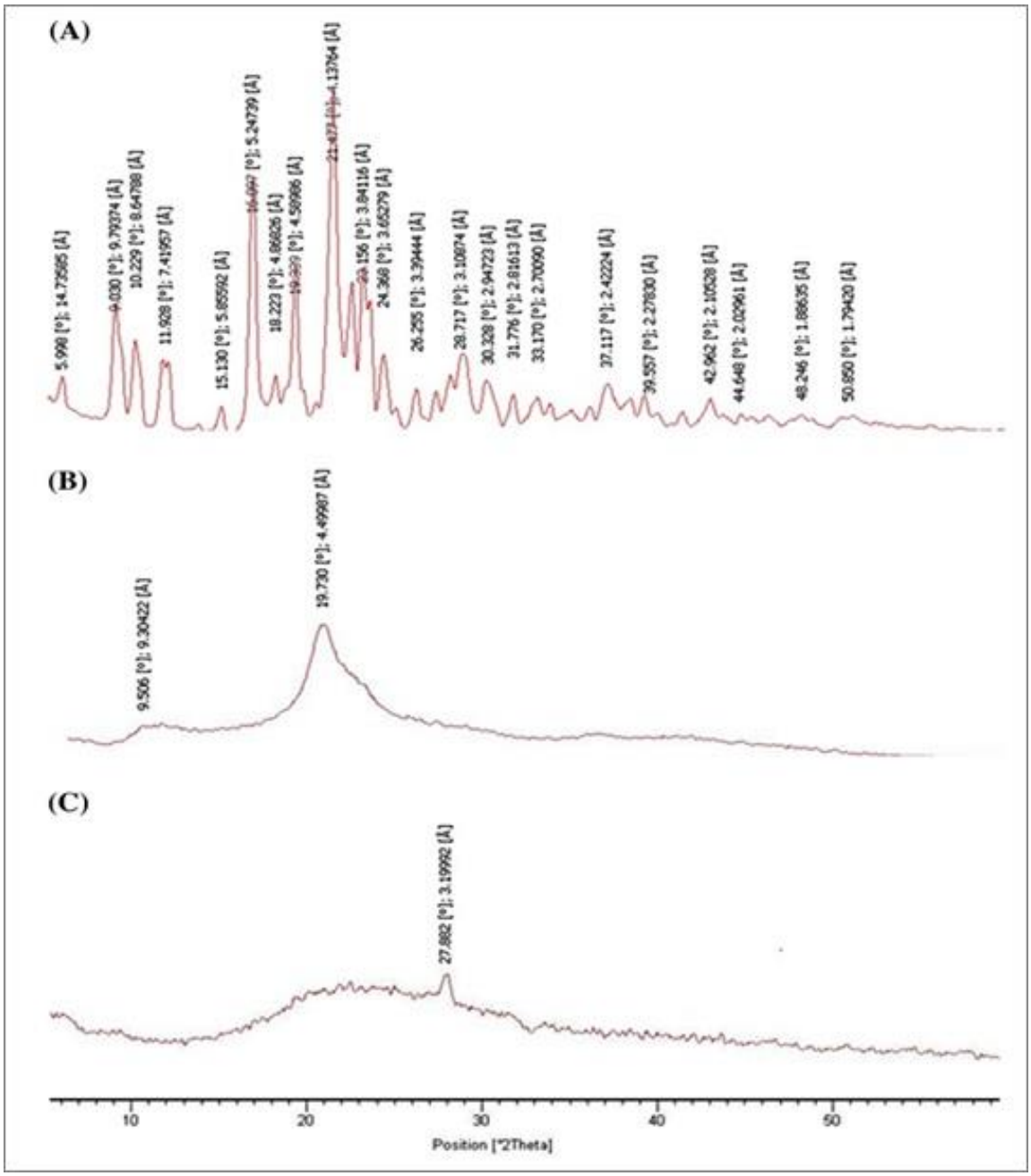

Fig. 4: XRD spectra of RS (A), chitosan (B), CH–RS complex (C)

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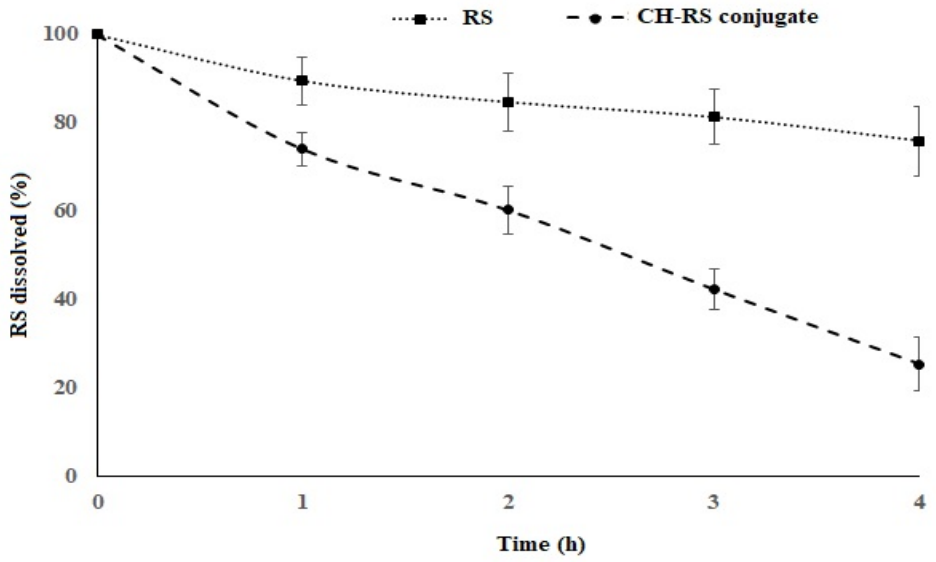

Fig. 5: Acid-degradation kinetics

Evaluation of muco-adhesive properties of CH–RS complex

The binding responses of pure RS and CH–RS complex on intestinal membrane after 4 h were found to be 2.6% and 48.5%, respectively. The muco-adhesive nature of the CH–RS complex was due to the presence of CH, which is known as a muco-adhesive. This result suggests clearly that CH–RS complex retains the muco-adhesiveness of the parent CH, which was used to prepare the complex. We also speculate that low muco-adhesiveness as observed for pure RS might be due to its entrapment in the mucin layer of the intestine.

Solubility studies

The aqueous solubility of pure RS and CH–RS complex was found to be 0.1 and 25.0 mg/mL, respectively. As expected, the solubility of CH–RS complex was approximately 250-fold higher than that of pure RS. This improved aqueous solubility of the RS for CH–RS complex could be attributed to the collective effect of the formation of water soluble complex and also due to amorphous RS in CH–RS complex.

Acidic degradation kinetic studies

It is well known that RS, like other statins, is unstable and undergoes acid-catalyzed degradation to form lactones. The study was performed to determine ability of CH–RS complex to prevent the acid-catalyzed degradation of RS. The Fig. 5 shows the degradation kinetics of RS and CH–RS complex. The results clearly indicate that formation of complex stabilizes the drug. At 4 h time point, for RS only 25.5 ± 6.1 % drug remains, whereas at similar conditions for CH–RS complex 75.9 ± 7.8 % drug remains. The results demonstrated nearly 3-fold higher stability of RS from complex formulation. This probably could be due to the protection of carboxylic group of RS through complexation with the amine group of CH.

Radical-scavenging activity (RA)

DPPH is a stable nitrogen-centered free radical commonly used for testing radical scavenging activity of the compound or plant extracts. When the stable DPPH radical accepts an electron from the antioxidant compound, the violet color of the DPPH radical reduced to yellow colored diphenylpicrylhydrazine radical, which is measured colorimetrically. Substances such as RS, which are able to perform this reaction, can be considered as radical scavengers and their radical scavenging activity is dependent upon the amount of concentration of RS in solution. Results in Fig. 6 showed that

CH-RS complex exhibited higher DPPH radical scavenging activity compared to the RS. This could be probably due to the high solubility and stability of complex which resulted in better radical scavenging activity.

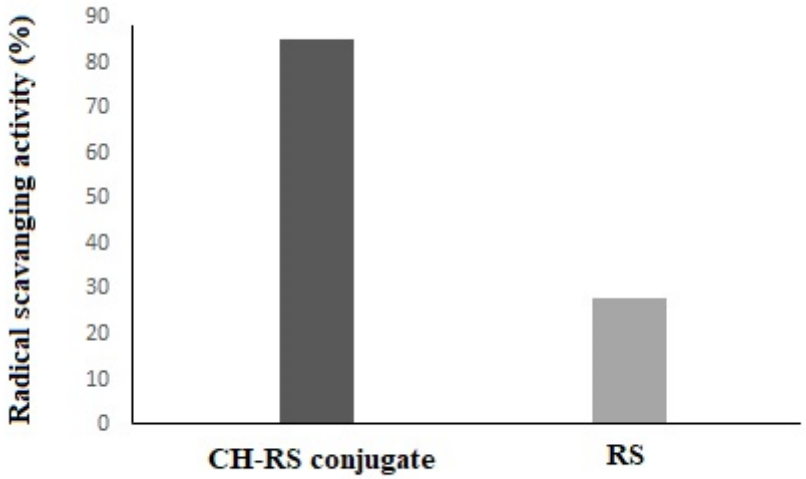

Fig.6: DPPH radical scavenging activity of the CH–RS complex compared to the RS

CONCLUSION

It has been observed that the chitosan-rosuvastatin complex retains the muco-adhesiveness of the parent chitosan; its solubility was approximately 250-fold higher than that of pure rosuvastatin; it demonstrated nearly 3-fold higher stability than the rosuvastatin; and it exhibited higher DPPH radical scavenging activity compared to the rosuvastatin. Accordingly, it has been concluded that the chitosan-rosuvastatin complex has a potential to demonstrate enhanced bioavailability *in vivo*. Therefore, further *in vivo* studies are recommended.

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REFERENCES

[1] Cheng JW. Rosuvastatin in the management of hyperlipidemia. Clinical Therapeutics, 2004; 26(9): 1368-1387.

[2] Milionis HJ, Gazi IF, Filippatos TD, Tzovaras V, Chasiotis G, Goudevenos J, Seferiadis K, Elisaf MS. Starting with rosuvastatin in primary hyperlipidemia - Is there more than lipid lowering?, Angiology, 2005; 56: 585-592.

[3] Rosenson RS. Rosuvastatin: a new inhibitor of HMG-coA reductase for the treatment of dyslipidemia. Expert Review of Cardiovascular Therapy, 2003; 1(4): 495-505.

[4] Olsson AG, McTaggart F, Raza A. Rosuvastatin: a highly effective new HMG-CoA reductase inhibitor. Cardiovascular Drug Reviews, 2002; 24(4): 303-328.

[5] Adams SP, Sekhon SS, Wright JM. Lipid-lowering efficacy of rosuvastatin. The Cochrane Database Systemic Reviews, 2014: CD010254. doi: 10.1002/14651858.CD010254.pub2.

[6] Scott LJ, Curran MP, Figgitt DP. Rosuvastatin: a review of its use in the management of dyslipidemia. American Journal of Cardiovascular Drugs, 2004; 4(2): 117-138.

[7] Birmingham BK, Swan SK, Puchalski T, Mitchell P, Azumaya C, Zalikowski J, Wang Y. Pharmacokinetic and pharmacodynamic profile of rosuvastatin in patients with end-stage renal disease on chronic haemodialysis. Clinical Drug Investigation, 2013; 33(4): 233-241.

[8] McKenney JM. Efficacy and safety of rosuvastatin in treatment of dyslipidemia. American Journal

Health-system Pharmacy, 2005; 62(10): 1033-1047.

[9] Feng J, Chen Y, Li F, Cui L, Shi N, Kong W, Zhang Y. Synthesis, characterization and i*n vitro* evaluation of a novel glycol chitosan-EDTA conjugate to inhibit aminopeptidase-mediated degradation of thymopoietin oligopeptides. Molecules, 2017; 22(8). pii: E1253. doi: 10.3390/molecules22081253.

[10] Fan Y, Yi J, Zhang Y, Yokoyama W. Improved chemical stability and antiproliferative activities of curcumin-loaded nanoparticles with a chitosan chlorogenic acid conjugate. Journal Agricultural Food Chemistry, 2017; 65(49): 10812-10819.

[11] Wang JJ, Zeng ZW, Xiao RZ, Xie T, Zhou GL, Zhan XR, Wang SL. Recent advances of chitosan nanoparticles as drug carriers. International Journal Nanomedicine, 2011; 6: 765-774.

[12] Benediktsdóttir BE, Baldursson Ó, Másson M. Challenges in evaluation of chitosan and trimethylated chitosan (TMC) as mucosal permeation enhancers: From synthesis to *in vitro* application. Journal Control Release, 2014; 10(173): 18-31.

[13] Anwar M, Warsi MH, Mallick N, Akhter S, Gahoi S, Jain GK, Talegaonkar S, Ahmad FJ, Khar RK. Enhanced bioavailability of nano-sized chitosan-atorvastatin conjugate after oral administration to rats. European Journal Pharmaceutica Sciences, 2011; 44(3): 241-249.

[14] Akter S, Ahmed M, Eun JB. Solvent effects on antioxidant properties of persimmon (Diospyros kaki L. cv. Daebong) seeds. International Journal Food Science Technology, 2010; 45: 2258-2264.