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Controlled Ondansetron Release Based on Hydroxyethyl Starch Hydroxyethyl Methacrylate

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Presented study describes the synthesis of photo cross-linkable and water soluble hydroxyethyl starch hydroxyethyl methacrylate (HESHEMA) samples with different degree of substitution (DS) by functionalization of hydroxyethyl starch (HES) with hydroxyethyl methacrylate (HEMA) or hydroxyethyl methacrylate carbonylimidazole (HEMACI) in DMSO using two different routes. It was revealed that the reaction time for HESHEMA synthesis can be reduced from 5 days to 24 h by conducting the reaction at 80 °C instead of at room temperature. Solubility of HESHEMA was found to be dependent on DS which in turn was dependent on ratio between HES and HEMA or HEMACI. HESHEMA samples with DS > 0.24 depicted insoluble in water, whereas the samples with DS < 0.05 did not form appreciable gel. HESHEMA samples with appropriate DS were converted into hydrogels by cross-linking polymer chains under UV radiations and resulting HESHEMA hydrogels showed swelling up to 1200%. Application of HESHEMA in controlled drug delivery was investigated by diffusion based encapsulation of Ondansetron, a serotonin 5-HT_3 receptor antagonist drug, mainly used for nausea and vomiting treatment.

Key Words : Hydroxyethyl starch, Hydrogels, Ondansetron, Controlled drug release

Introduction

Hydrogels are the topic of interest for biomaterial researchers due to their specific characteristics *e.g.* biocompatibility, biodegradability and hydrophilicity.[1,2] Hydrophilic properties of hydrogels are important because hydrophilic parts of the hydrogels get hydrated in water, triggering big amount of water uptake resulting into hydrogel swelling. Swelling ratio of hydrogels can be enhanced up to many times of their dry weight[3,4] and depends mainly upon degree of substitution (DS) of cross-linking substituents, pH and nature of biopolymer used.

During the last few decades, lot of efforts have been devoted by the scientific community to design new materials that can be used for control delivery of drugs. Moreover, increasing trends regarding the applications of peptides and proteins in drugs increases the need of new materials for drug delivery systems (DDS). Hydrogels due to their biocompatible nature can fulfil these requirements. Such a DDS should be able to deliver an active substance (drug) to a specific site while keeping its concentration on an optimal level required for its effectiveness for a specific period of time. Biocompatibility is another basic requirement for such DDS to avoid adverse effects along with their elimination from the body within a limited period of time.[4] Along with biocompatibility and biodegradability, the size and shape of DDS are also important for the release of drug at a specific site.

Biopolymers, due to their biocompatibility and biodegradability are widely used for synthesis of hydrogels.[4] Hydroxyethyl starch (HES), a branched polysaccharide with α(1-4) glycosidic linkage and branching at α(1-6) positions, due to its biocompatibility and biodegradability, is already being used in many pharmaceutical applications.[5] Biocompatible and biodegradable hydrogels can be prepared by cross-linking of such biopolymers after suitable chemical modifications. During the current research work, hydroxyethyl methacrylate groups were grafted on HES backbone by etherification reaction. Most probably, substitution occurs at free hydroxyl groups available at position C-2, C-3 and C-6 but can also be executed at already introduced hydroxyethyl groups.[6] Solubility and biodegradability of HES derivatives depends not only upon DS (decreases by increasing the DS) but also upon molar substitution (MS) and ratio of substitution at C-2 to C-6.[5] Degree of substitution is defined as the average number of substituted hydroxyl groups per Anhydrous Glucose Unit (AGU) and in case of HES, it can range from 0-3.[7] On the other hand, Molar Substitution (MS) is defined as the average number of substituents per AGU and theoretically have no upper limit and can be executed with much higher levels comparative to DS.[8]

Releasing behaviour of encapsulated drug(s) usually depends upon lability of chemical bonds in cross-links under the physiological conditions used.[9] Different techniques *e.g.* ionic interactions,[10] crystallization,[11] use of enzymes,[12] chemical reactions of complementary groups,[13,14] addition reactions,[15] condensation reactions,[16] polymerization of substituent on biopolymers[17] and radiation methods[18-21] have been developed to achieve cross-linking for the preparation of hydrogels. Among these radiation methods are revealed to

be most attractive being easy, simple and capable of cross-linking in water under mild conditions (room temperature, physiological pH). Normally cross-linking density directly depends upon polymer concentration and radiation dose and increases with increase in both of these factors.[9]

In this study we reported the synthesis of hydrogels based on modified HES and encapsulation of Ondansetron, a serotonin 5-HT_3 receptor antagonist drug. This drug is mainly used as an antiemetic (to treat nausea and vomiting) drug often following chemotherapy. Some recent studies have shown that ondansetron might be useful and effective for treating withdrawal symptoms of opioid addictions,[22] Ondansetron lowers the cravings for alcohol, especially in early-onset alcoholics[23] and is also effective for postanesthetic shivering, commonly occurring after surgery.[24]

Experimental

Materials. Hydroxyethyl starch (HES, M_w, 200000 $gmol^{-1}$, DS 0.4), hydroxyethyl methacrylate (HEMA, ≥ 99%), 1,1-carbonyldiimidazole (CDI, ≥ 97%), THF (≥ 99.9%, anhydrous), and ethylacetate (99.8%) were purchased from Sigma-Aldrich. Dimethyl sulfoxide (DMSO, 99.7%) was purchased from Acros Organics. Photoinitiator (Igracure 2959, 97-99.5%) was procured from Ciba, whereas D_2O (99.9%) from Aldrich. THF was dried before use and ethyl acetate was distilled, while all other chemicals were used without further purification or treatment.

Synthesis of HEMACI. HEMA (7.22 g, 55.5 mmol) and CDI (9.02 g, 55.5 mmol) were added in a dried flask containing DMSO (90 mL) under nitrogen stream. Clear solution was formed within few minutes and the mixture was stirred at room temperature. After 16 h, reaction was inhibited by adding *p*-cresol (2.70 g, 25 mmol). Solvent was evaporated under vacuum. Crude product was dissolved in ethyl acetate (50 mL) and extracted with water to remove imidazole and other un-reacted reagents. Organic phase was dried over anhydrous $MgSO_4$ and filtered. Ethyl acetate was evaporated under vacuum and HEMACI (11.50 g, 50.87 mmol, 92%) was obtained as light yellow oil.

Synthesis of HESHEMA. To get a range of DS, different ratios, ranging from HES/HEMACI 1/0.33 to 1/1.0 were applied. In a typical reaction, 5.43 g (30.5 mmol) of HES and 1.09 g (9.0 mmol) of DMAP were dissolved in DMSO (100 mL) in a dry flask under nitrogen at room temperature. After 1 h, 9.17 g (30.5 mmol) of HEMACI was added drop wise to the mixture. Temperature was raised to 80 °C with stirring that continued for next 24 h. Samples (HESHEMA) were withdrawn from reaction mixture at specific intervals, dialyzed against deionised water and freeze-dried to get whitish fluffy HESHEMA.

DS Measurements of HESHEMA. 40 mg sample was dissolved in 1 mL D_2O. Bruker Type AM 400 spectrometer was used to record all ^{1}H-NMR spectrum. For DS determination from ^{1}H-NMR, intensity of signal corresponding to anomeric proton (proton of C-1, 5.25-6.1 ppm) was compared with intensity of signal corresponding to terminal methyl group protons of HEMA substituent (1.66-2.16 ppm).

Infrared Spectroscopy. 2 mg of HESHEMA sample was mixed thoroughly with 150 mg of extra pure KBr and was converted into a thin solid film under high pressure. FT-IR-Equinox IFS spectrometer equipped with OPUS-spectroscopic (version 4) was used to record ATR-IR spectrum using air as reference for all samples.

Cross-linking of HESHEMA (hydrogel formation). 100 mg of HESHEMA (samples with different DS) was dissolved in 900 mg of 0.1% aqueous photoinitiator (Irgacure-2959) solution. 2 mL of this solution was then added in a vial for cross-linking. Three hydrogels were prepared from each sample. Ultra violet (UV) lamp (366 nm, 3.5 $mW{\cdot}cm^{-2}$) was placed 3 mm above the vials containing HESHEMA solution for 30 min. The resulting hydrogel cylinders were soaked in water before freeze-drying. To study the swelling behaviour of hydrogels, dried hydrogels were weighed and socked in deionised water. To monitor the swelling progress, the hydrogels were weighed periodically after removing the surface water from hydrogels with a paper towel. This process was repeated, until constant weight was gained. The swelling ratio of the hydrogels was calculated according to the following equation.[25]

$$\text{Swelling Ratio (\%)} = Q_M = \frac{M_s - M_d}{W_d} \times 100 \qquad (1)$$

M_s is the mass of the swollen hydrogel while M_d is the mass of the dried hydrogel.

Drug Loading. Four different batches of hydrogels (using HESHEMA of different DS) having six samples in each batch were prepared by the method described above. Ondansetron (Figure 1) was loaded into the hydrogels by a swelling-diffusion technique. Dried and weighed hydrogels were immersed in 10 mL of drug solution (1 mg/mL) and allowed to swell for 1 day at room temperature. After swelling equilibrium reached, hydrogels were taken out of the solution and washed with water to remove non-encapsulated drug. Remaining concentration of the solution was measured by UV-visible spectrophotometer (Jasco V-530) and the drug loaded into the hydrogels was calculated from the difference of initial and after absorption concentration of the solution.

***In vitro* Drug Release Studies.** To study the releasing behaviour of encapsulated drug, hydrogels were dialysed (MWCO 12000-14000 Da) against phosphate buffer solution (PBS, pH 7.4, 10 mM) (0.01% w/v sodium azide) at 37 ºC. The quantification of drug released as a function of time was monitored by using same spectrophotometer. Samples were taken every day from the acceptor reservoir. The acceptor medium was refilled daily with the same amount of fresh

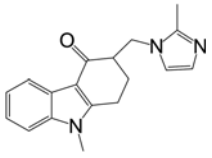


Figure 1. Chemical structure of Ondansetron drug used in this study.

buffer.

Results and Discussions

Synthesis of HESHEMA. HES backbone was grafted with HEMA by two different routes (Scheme 1) to determine most effective route regarding better product based upon DS and solubility in water. Same reagents and reaction conditions were used in both cases but the order of addition of reagents was different. In route 1, HEMA was reacted with CDI to synthesize HEMACI, which in next step was grafted on HES to prepare HESHEMA. In route 2, HES was first reacted with CDI to form HESCI, which in second step was reacted with HEMA to prepare HESHEMA. Product in both cases was purified by dialysis against deionised water (MWCO 12000-14000). During dialysis and also afterwards from DS determination, it was observed that HESHEMA prepared by route 1 showed better solubility in water as compared to HESHEMA prepared by route 2 with comparable DS. The presence of imidazole groups in route 2 comparative to unreacted hydroxylethyl (and hydroxyl) groups in route 1 may be the possible reason regarding the decreased solubility of resulting HESHEMA synthesized by route 2. Harling *et al.* and Schwoerer *et al.*[4,26] synthesized HESHEMA by route 1 and reactions were completed by stirring HEMACI and HES for 5 days at room temperature. Here in this study, after a series of optimization reactions of HEMACI and HES conducted at different temperature conditions, it was found that reactions executed at 80 ºC for 24 h (Scheme 1) gave almost same DS as reported by other researchers.[4,26]

HESHEMA samples using different ratio of HEMACI and HES (route 1, Scheme 1) were prepared. To get HESHEMA with highest possible DS but keeping it soluble in water, HESHEMA samples were withdrawn from reaction vessel at specific intervals (Figure 2), dialyzed against deionized water, freeze-dried and characterized by ATR-IR and ^{1}H-NMR spectroscopy. All samples showed a strong absorbance at 1750 cm^{-1} corresponding to ester group in HESHEMA after substitution. The dried HESHEMA samples were converted into hydrogels by using a photo initiator (Irgacure 2959) and UV irradiation as described in experimental section.

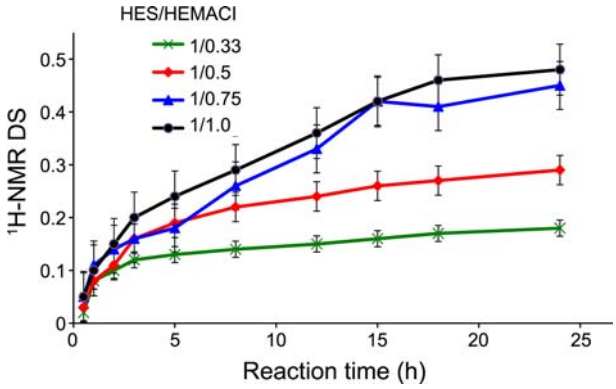
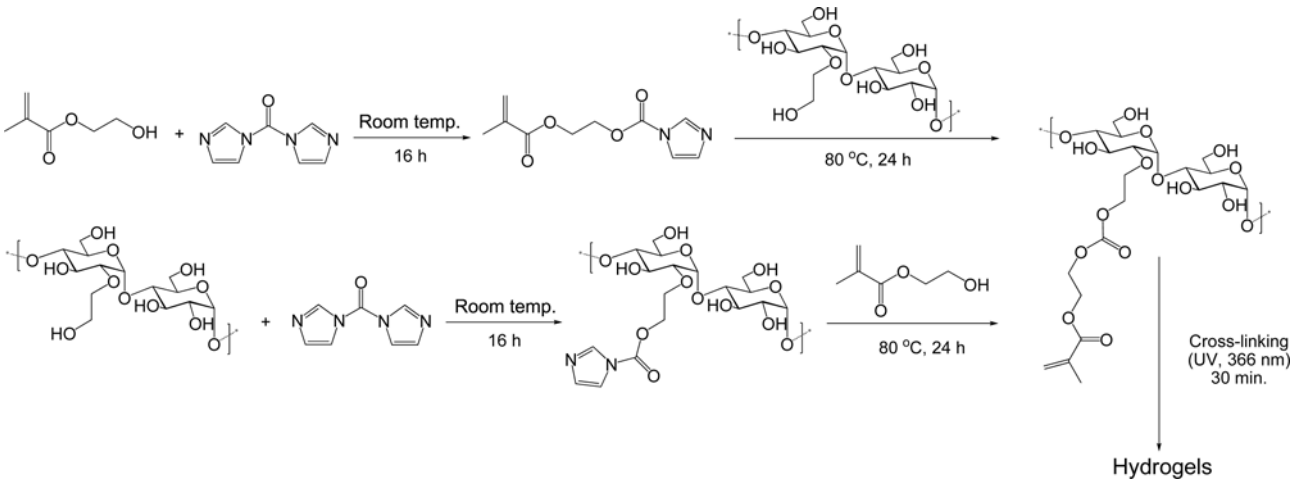


Figure 2. Relationship between ratio of HES/HEMACI, reaction time and degree of substitution.

HES is water soluble due to hydrogen bonding between free -OH groups of HES and water. During formation of HESHEMA, as substitution reaction proceeds, -OH of HES decreases as DS increases, resulting into decrease in its solubility in water. On the other hand, DS depends upon molar ratio of HEMACI and HES along with reaction time (keeping the temperature constant) *i.e.* normally DS increases (up to a certain limit) with increasing reaction time. The number of cross-linkable HEMA groups attached per AGU (DS) is key to adjust desirable cross-linking density and resultantly drug releasing properties of formed hydrogels. To adjust all these properties, the HES/HEMACI ratio was varied and samples were withdrawn periodically (Figure 2).

HESHEMA samples from all batches were taken periodically and DS was calculated. From Figure 2, it is clear that DS depends upon ratio of HES/HEMACI and reaction time and increases up to a certain limit with increase in the reaction time. HES/HEMACI ratio was varied from 1/0.3, to 1/1.0 in four steps. Samples with higher HEMACI concentration gave higher DS comparative to the samples with lower HEMACI during the same reaction time. Cross-linking (gel formation) of HESHEMA was found to be strongly dependent upon DS. It was observed that samples with DS 0.24 or



Scheme 1. Synthesis of hydroxyethyl starch hydroxyethyl methacrylate (HESHEMA) based hydrogels.

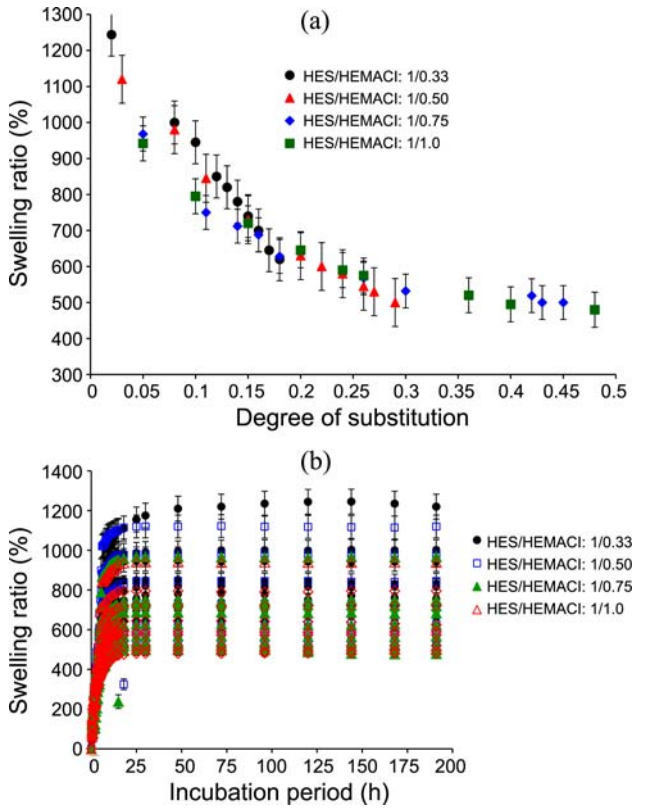


Figure 3. Relationship between swelling ratio and (a) degree of substitution (b) incubation period for hydrogels formed from HESHEMA.

higher were precipitated in water during dialysis, whereas HESHEMA samples with DS 0.05 or lower gave very poor or almost no gel formation. Therefore the samples with DS range (0.08 to 0.22) were converted to hydrogels using radiation method as described above and were used for further studies.

Swelling Measurements of Hydrogels. Hydrogels were prepared from dried HESHEMA samples using photoinitiator (Igarcure 2959) under the exposure of UV radiations for 30 minutes. After gel formation, samples were weighed and dried under vacuum. To study swelling behaviour, dehydrated hydrogels were immersed in PBS at pH 7.4 and 37 °C. Swollen gels were taken out of the medium at regular intervals, dried superficially with filter papers, weighed and placed in the same medium. These measurements were continued until a constant weight was reached for all samples. HESHEMA hydrogels with different DS, prepared from different ratio of HES and HESCI showed swelling ratio from 500 to 1200%. Depicted relation between DS and swelling ratio is shown in Figure 3(a).

The main driving force for water to enter into hydrogels is the osmotic pressure, while elastic force of the stretched polymer network counteracts the swelling process of hydrogels. Equilibrium degree of swelling is achieved, when these two forces become equal. Amount of buffer absorbed in hydrogels was then calculated by measuring the hydrogel weight for up to 191 h. Maximum increase in weight was observed in first 25 h (Figure 3(b)). After about 150 h, weight of certain synthesized hydrogel samples was found to be decreased, most probably due to partial degradation in water or due to degradation in samples during handling and weighing.

Calculation of Average Molecular Weight Between Cross-links ($\overline{M_c}$). Average molecular weight between cross links ($\overline{M_c}$) was calculated according to Flory-Rehner equation.[27]

$$\overline{M_c} = \frac{Q_v^{5/3} \times V_1}{(1/2 - \chi_1) \times \overline{\upsilon}} \quad (2)$$

Where Q_v is the volumetric swelling ratio, V_1 is the molar volume of the solvent (18 for water), χ_1 is the Flory polymer-solvent interaction parameter and $\overline{\upsilon}$ is the specific volume of the dry polymer.

Q_v in Eq. (2) was calculated by applying Eq. (3).[27]

$$Q_v = 1 + \frac{\rho_p}{\rho_s}(Q_M - 1) \quad (3)$$

In Eq. (3), ρ_p is density of the dry polymer. To make it simple, ρ_p for dextrin (1.66667 g/cm^3) was used in current study. ρ_s is the density of the solvent (water, 1.0 g/cm^3). Q_M is the hydrogel swelling ratio and was determined by dividing the gel mass after swelling (M_s) by the dry gel mass (M_d) as described in Eq. (1).

It was revealed that swelling ratio depends upon $\overline{M_c}$ which in turn depends upon average DS (Figure 4). Samples with higher DS showed higher cross-linking, leading to lower $\overline{M_c}$ and lower swelling ratio (Figure 3(a)).

Drug Release Behaviour from Hydrogels. Drug release studies from cross-linked HESHEMA for all samples were carried in PBS (pH 7.4) at 37 °C. Amount of the drug released from HESHEMA hydrogels was quantified by UV spectrophotometer using a validated calibration curve at 312 nm. Calculations, as described in the experimental section showed that the total amount of drug encapsulated in cross-linked HESHEMA varied from 2.005 to 2.261 mg/g of cross-linked polymer material. Variation of drug encapsulation was probably due to different DS of HESHEMA samples used in this study. It is already reported that higher DS in HESHEMA leads to higher cross-linking which results into lower $\overline{M_c}$ (see Figure 4) and small pore size. Therefore,

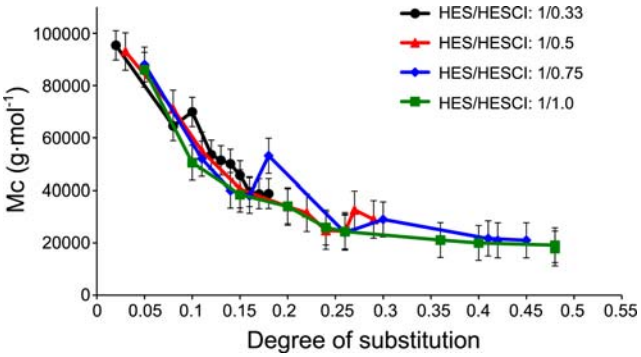


Figure 4. Relationship between average molecular weight between cross-links ($\overline{M_c}$) and degree of substitution (DS) in hydrogels formed from HESHEMA.

Table 1. Relation between DS and drug (Ondansetron) loading capacity for HESHEMA hydrogels

DS	Drug loading (mg/g of biopolymer)
0.22	2.261
0.16	2.151
0.11	2.102
0.08	2.005

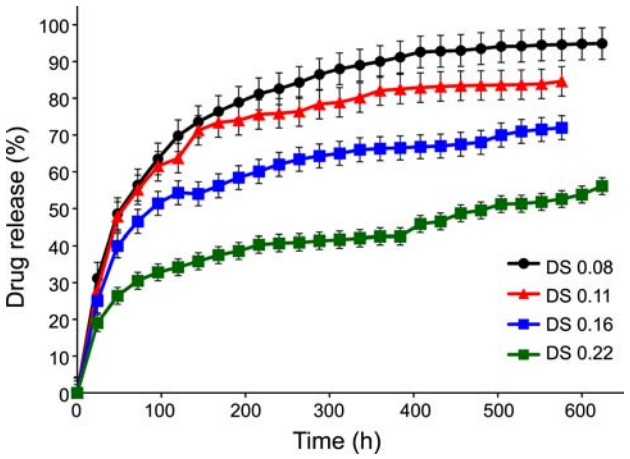


Figure 5. Release of Ondansetron drug from cross-linked HESHEMA in phosphate buffer solution (10 mM) at pH 7.4 and 37 ºC.

drug loading capacity depends upon DS and decreases with increase in DS. The relationship between DS and drug loading capacity for HESHEMA is shown in Table 1.

Data for drug release as function of time is presented in Figure 5. All data is presented as a percentage of the initial amount entrapped in the hydrogel. Figure 5 shows the releasing behaviour of all four batches at 37 ºC. All entries are the mean values of three measurements for each batch.

Release behaviour of all four batches of HESHEMA hydrogels was different and depicted that release behaviour of Ondansetron was dependent strongly upon DS of cross-linkable substituents on the biopolymer. The drug release started with very fast burst followed by a slower phase with almost constant liberation. Initial fast release was probably due to release of drug stored in hydrogel pores or due to release of drug molecules closer to the surface, while slower release in second phase occurred by diffusion of drug molecules through the polymer network.[4,21] This phenomenon of fast release followed by a slow release of encapsulated drug is also reported by other researchers for the same or different drugs.[4,28,29] It was already discussed during the study related to the study of average molecular weight between cross-links ($\overline{M_c}$) that $\overline{M_c}$ decreases as DS increases (Figure 4). Thus higher DS (*i.e.* lower $\overline{M_c}$) leads to higher cross-linked networking, resulting into more tight retention of drug inside the gel matrix. Slower release of drug after a phase of fast release was probably due to diffusion limitations of Ondansetron through hydrogel matrix. It was supposed that increase in release rate after about 400 h incubation (especially for sample with DS 0.22) was probably due to degradation of hydrogels during handling although macroscopic degradation of hydrogels was not observed.

Conclusions

We have prepared hydrogels based on polymerization of hydroxyethyl methacrylate substituents grafted on backbone of hydroxyethyl starch biopolymer. Solubility of modified hydroxyethyl starch (HESHEMA) was found to be influenced by the order of addition of the reagents (see route 1 and route 2 in Scheme 1). DS of substituent in HESHEMA depends upon relative ratio of HES/HEMACI and reaction time. Solubility of HESHEMA was also found to be dependent upon DS of substituents. Average molecular weight between cross-links ($\overline{M_c}$) depends strongly upon DS of HESHEMA used and decreases with increase in DS. Ondansetron drug was encapsulated in HESHEMA after gel formation. Release of drug from hydrogels showed fast release in first few hours followed by slower long term release of Ondansetron by diffusion process. Most probably biodegradable biopolymers will degrade fast in *in vivo* experiments resulting into fast drug release.

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