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Enhanced drug loading efficiency of contact lenses via salt-induced modulation

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ABSTRACT

Low drug loading efficiency is one of the main obstacles hindering the application of contact lenses (CLs) as the carrier for extended ocular drug delivery. Here in this study, a simple and effective drug loading method based on salt induced modulation was proposed and demonstrated with mechanism elucidation. First of all, using poly (2-hydroxyethyl methacrylate) (p-HEMA) as the contact lens material, betaxolol hydrochloride, Diclofenac Sodium and Betaxolol Base as the model drugs with different solubility, influence of salt concentration, salt type (sodium salts of sulfate, chloride, and sulfocyanate) and drug properties in the loading solution on drug loading efficiency was investigated. Mechanism of enhanced drug loading in contact lens was further explored via studying the influence of salt on the absorption isotherm, drug solubility and water content of CLs. Applicability of this method to other CLs materials was also investigated. It was demonstrated that adjusting the ionic strength of loading solutions resulted in significant increase of drug loading in CLs. Type and concentration of the salts and solubility of the drug were the main factors influencing enhancement ratio of drug loading. The mechanism for improved drug loading was related to the reduced drug solubility in loading solutions and the reduced bound water content in contact lenses. Modulation of drug loading by adjusting ionic strength was also applicable to other CLs and the light transmittance was not affected. This method was more suitable for salt-form drugs with high solubility. In summary, adjusting ionic strength of loading solution is an economical and effective way to improve drug loading in CLs, and this simple method may also find application in other hydrogel based drug delivery systems.

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1. Introduction

Topical delivery is the most accepted route of ocular drug application for its simplicity and affordable price. It is often used to treat anterior segment diseases such as glaucoma, dry eye, trachoma, although some studies have proved that drugs can reach posterior segment of the eye through topical route [1]. At present approximately 90% of aqueous ophthalmic formulations are used in this way. However, drugs instilled as eye drops usually have a low bioavailability less than 5% due to

the existence of static and dynamic barriers [2], therefore frequent dosing is required. At this stage almost 75% of the eye drops on the market are recommended to use 3 times daily or even more, which is a great challenge to the compliance of patients.

So far, several strategies have been used to prolong drug retention in the precorneal area, such as viscosity-enhancing polymers [3], in-situ gels [4,5], mucoadhesive system [3], ocular inserts [6] and soft contact lenses [7]. In order to increase drug permeation across the cornea, nanocarries [8], penetration enhancers [9] and solubility enhancers [10] are commonly used, with improved drug ocular bioavailability. Among them, contact lenses (CLs) have attracted extensive attention due to greatly improved ocular retention time compared with other methods. In 1965 contact lens was firstly used in ocular drug delivery and since then numerous studies have proved that contact lens is more effective than eye drops [7,11,12]. However, no medicated contact lenses are available in the market until now and low drug loading is the primary obstacle hindering further development of CLs in the field of drug delivery. For drug loading in CLs, soaking and pre-incorporating are two commonly used methods. Pre-incorporating is to introduce drugs before CLs being formed, which is often used in nanocarriers laden CLs [13]. Soaking is the conventional method with CLs immersed in drug solution, while most of the drug in the soaking solution is not loaded by the CLs and drug waste is a problem [12]. To improve drug loading of CLs, several attempts have been carried out including accommodation of CLs composition, molecular imprinting [14], incorporation of cyclodextrins [15], multilayer CLs [16]. Those strategies have achieved some progress, however, incomplete drug release and lens deformation in CLs copolymerized with ionizable monomers [17], modulus increase in hydrophobic silicone [18] and cyclodextrins CLs [15], high crosslinker in molecular imprinting CLs [19] are undesired. Therefore, a simple and effective drug loading method without altering lens compositions are highly desirable.

Unexpectedly, during our preliminary experiment, fivefold increase of diclofenac sodium loading in poly (2-hydroxyethyl methacrylate) (pHEMA) lens was found when simulated tear fluid (STF) was used as the soaking solvent compared to that using distilled water as solvent. Thereafter, different salt solutions were used as loading solvent and it was found that drug loading in all of those salt solutions were improved compared with that of distilled water. However, whether this is a general rule for other types of CLs or drugs, and the mechanism for increased drug loading by salt addition are unclear and needs to be elucidated. Meanwhile, detailed characterization of salt influence on the properties of CLs is essential.

Therefore, the main objectives of this study are (1) to understand the mechanisms of drug loading increase via increasing ionic strength of loading solution; (2) to investigate the influence of salts addition on the properties of CLs. Both hydrophilic and hydrophobic drugs, Diclofenac Sodium (DS), Betaxolol Base (BB) and Betaxolol Hydrochloride (BH) were selected as drug model in this study. According to Hofmeister series, sodium salts of sulfate, chloride, and sulfocyanate were chosen to investigate the effect of salts on drug solubility and CLs loading.

## 2.	Materials and methods

### 2.1.	Materials

2-hydroxyethyl methacrylate (HEMA), methacrylic acid (MAA), 4-Vinylpyridine(VP), N-vinyl pyrrolidone 4-vinylpyridine (NVP), 3-methacryloxypropyl tris (trimethylsiloxy) silane (TRIS) were purchased from TCI (Tokyo, Japan). Ethylene glycol dimethacrylate (EGDMA) was obtained from Fortuneibo-tech Co., Ltd. (Shanghai, China). 2,2′-Azobis(2-methylpropionitrile) (AIBN) was supplied by Qingdao Kexin Materials and Technology Co., Ltd. (Qingdao, Shangdong, China). Diclofenac Sodium (DS) was from Hubei Prosperity Galaxy Chemical Co., Ltd. (Wuhan, Hubei, China). Betaxolol hydrochloride (BH) and Betaxolol Base (BB) were from BioChem Partner Co., Ltd. (Shanghai, China). All other chemicals were of analytical grade.

### 2.2.	Synthesis of contact lenses

CLs were synthesized by free radical solution polymerization of the monomer as reported previously [20]. Briefly, an appropriate amount of the crosslinker EGDMA was dissolved in HEMA to obtain a concentration of 0.5% (w/w). Then, the mixture was bubbled with nitrogen for 15 min to remove dissolved oxygen before the addition of 0.3% (w/w) AIBN (initiator). Both mixtures were injected into CLs molds to keep a swelling thickness of 100 µm. The molds were then placed in an oven for 12 h at 50 °C followed by 24 h at 70 °C. After polymerization, CLs were immersed in distilled water overnight to facilitate the separation of CLs from the molds. Thereafter, the CLs were immersed in distilled water for 3 d, replacing water 3 times daily to remove residual monomers. Finally, the CLs were dried at 40 °C under vacuum overnight to reach a constant weight.

### 2.3.	Preparation and characterization of drug loaded contact lens

Drug loaded contact lens were prepared using soaking method. Briefly, dry CLs were immersed in 1 ml aqueous drug solutions and were kept at 25 °C in dark for 3 d. Drug concentration before and after soaking procedure was determined spectrophotometrically at 276 nm for DS ($A = 0.0284C + 0.0108$, $r = 0.9994$), 272 nm for BH ($A = 0.0035C + 0.0171$, $r = 0.9995$), 272 nm for BB ($A = 0.0039C + 0.0172$, $r = 0.9994$) (UV-2000, UNIC Instrument Corp., Shanghai, China). The drug loading amount ($Q_{loading}$) and loading efficiency (LE) was calculated, respectively, by the mass balance according to the following equations:

$$Q_{loading} = \frac{(C_0 - C_e)V}{m} \tag{1}$$

$$LE(\%) = \frac{C_0 - C_e}{C_0} \times 100\% \tag{2}$$

where $Q_{loading}$ is the amount of drug adsorbed per gram of CLs (mg/g), C_0 is the initial drug concentration (g/l), C_e is the equilibrium drug concentration (g/l), m is the mass of CLs (g) and V is the volume of loading solution (l).

To facilitate the illustration of salt effect on drug loading of CLs, E_i, enhancement factor of salt (Eq. 3) was defined as the ratio of drug loading in salt solutions to drug loading in distilled water.

$$E_i = Q_{loading,salt}/Q_{loading,water} \tag{3}$$

where $Q_{loading, salt}$ and $Q_{loading, water}$ are the amount of drug adsorbed per gram of CLs (mg/g) using salt solution and distilled water as solvent, respectively.

2.4. Characterization of contact lenses

2.4.1. Water content of contact lenses
Dry CLs were weighed accurately and then immersed in distilled water or different salt solutions, at 25 °C for 3 d and the increase in weight was measured. Total water content (WC_{total}) of CLs was calculated according to Eq. 4.

$$WC_{total}\% = \frac{W_t - W_0}{W_0} \times 100 \tag{4}$$

where W_0 is the initial weight of the dry CLs; W_t is the weight of the swollen CLs.

The mass of bound water was obtained from the difference between the mass of total water and that of free water as described in Eq. 5. To measure the content of free water in contact lens, DSC studies were performed using a Mettler Toledo model with the STARe System (Mettler, Zurich, Switzerland). The samples (3–5 mg) were scanned in sealed aluminum pans under nitrogen atmosphere. DSC curves were studied over a temperature range of −20–10 °C at a constant rate of 3 °C/min. The content of free water was obtained according to Eq. 6.

$$WC_{bound}\% = WC_{total}\% - WC_{free}\% \tag{5}$$

$$WC_{free}\% = \frac{Q}{\Delta H\, W_0} \times 100 \tag{6}$$

where Q is the heat absorbed during the melting process; ΔH is the melting enthalpy of water, W_0 is the weight of dry lenses [21].

For both water content calculations, weight of dry lens, other than weight of swollen lens was used as denominator to reflect the relationship between hydrogel and solvent intuitively.

2.4.2. Light transmittance
Wet CLs were cut with a cork borer and the discs were put into a 96-well plate. The transmittance of CLs was measured on a SpectraMax M3 microplate reader ranging from 400 nm to 900 nm using SpectraMax Pro Software (Molecular Devices, Sunnyvale, USA) and water was used as blank control.

2.5. Absorption isotherms

Different concentrations of DS (0.1–2 g/l), BH (0.5–5 g/l) and BB (0.1–0.5 g/l) in distilled water or 0.15 mol/l NaCl solutions were used as loading solutions to obtain absorption isotherm in different contact lens. Equilibrium isotherm data were fitted into Linear (Eq. 7), and Freundlich (Eq. 8) absorption isotherm models.

$$Q_{loading} = KC_e \tag{7}$$

$$\log Q_{loading} = \log K_F + \frac{\log C_e}{n} \tag{8}$$

where $Q_{loading}$ is the amount of drug adsorbed per gram of CLs (mg/g), C_e is the equilibrium drug concentration (g/l). K, and K_F are the Linear and Freundlich isotherm constants, n is the Freundlich exponent factor [22].

2.6. Solubility study

Excess drugs were placed in 5 ml distilled water or salt solutions with different ionic strength (NaCl, Na$_2$SO$_4$, NaSCN at 0.005–1 mol/l) in a 10 ml test tube. Then the tube was put into a shaker water bath at 25 °C and agitated at 100 rpm. At specified time points, the suspension was centrifuged and the supernatant was filtered through a 0.45 μm Millipore membrane. The filtrate was then diluted and assayed for drug content. The empirical Setschenow equation with little modification (Eq. 9) was used to describe the influence of salts on drug solubility, as follows:

$$\text{Log}\left(\frac{S_0}{S_{app}}\right) = K_s I \tag{9}$$

$$I = \frac{1}{2}\sum_{i=1}^{n} c_i z_i^2 \tag{10}$$

where S_0 is the drug solubility in water, S_{app} is its solubility in salt solutions, K_s is the Setschenow constant, I is the ionic strength of the solution, c_i is the molar concentration of ion (mol/l) and z_i is the charge number of that ion. The K_s values are positive for salts that decrease solubility (i.e., salting-out) and negative for salts that increase solubility (i.e., salting-in) [23].

2.7. In vitro release

Drug release from the contact lens was investigated *in vitro* condition. STF (6.78 g/l NaCl, 2.18 g/l NaHCO$_3$, 1.38 g/l KCl, 0.084 g/l CaCl$_2$·2H$_2$O) was used as the release medium. Briefly, the CLs were rinsed with distilled water after loading and dried with absorbent paper in order to remove residual drug on the surface. Then the CLs were immersed in 10 ml of release medium at 35 °C, under stirring (100 rpm). At predetermined time intervals, 2 ml aliquots of the supernatant were withdrawn for drug content measurement and meanwhile, the same volume of fresh release medium was added. Drug release studies were carried out in triplicate for each formulation tested and standard deviations were calculated. The difference in dissolution profiles was evaluated using similarity factor (f_2), which can be calculated using Eq. 11 [24].

$$f_2 = 50\log\left\{\left[1 + \left(\frac{1}{n}\right)\sum_{t=1}^{n}(R_t - T_t)^2\right]^{-0.5}\right\} \times 100 \tag{11}$$

where n is the number of time points, R_t is the dissolution value of the reference at time t, and T_t is the dissolution value
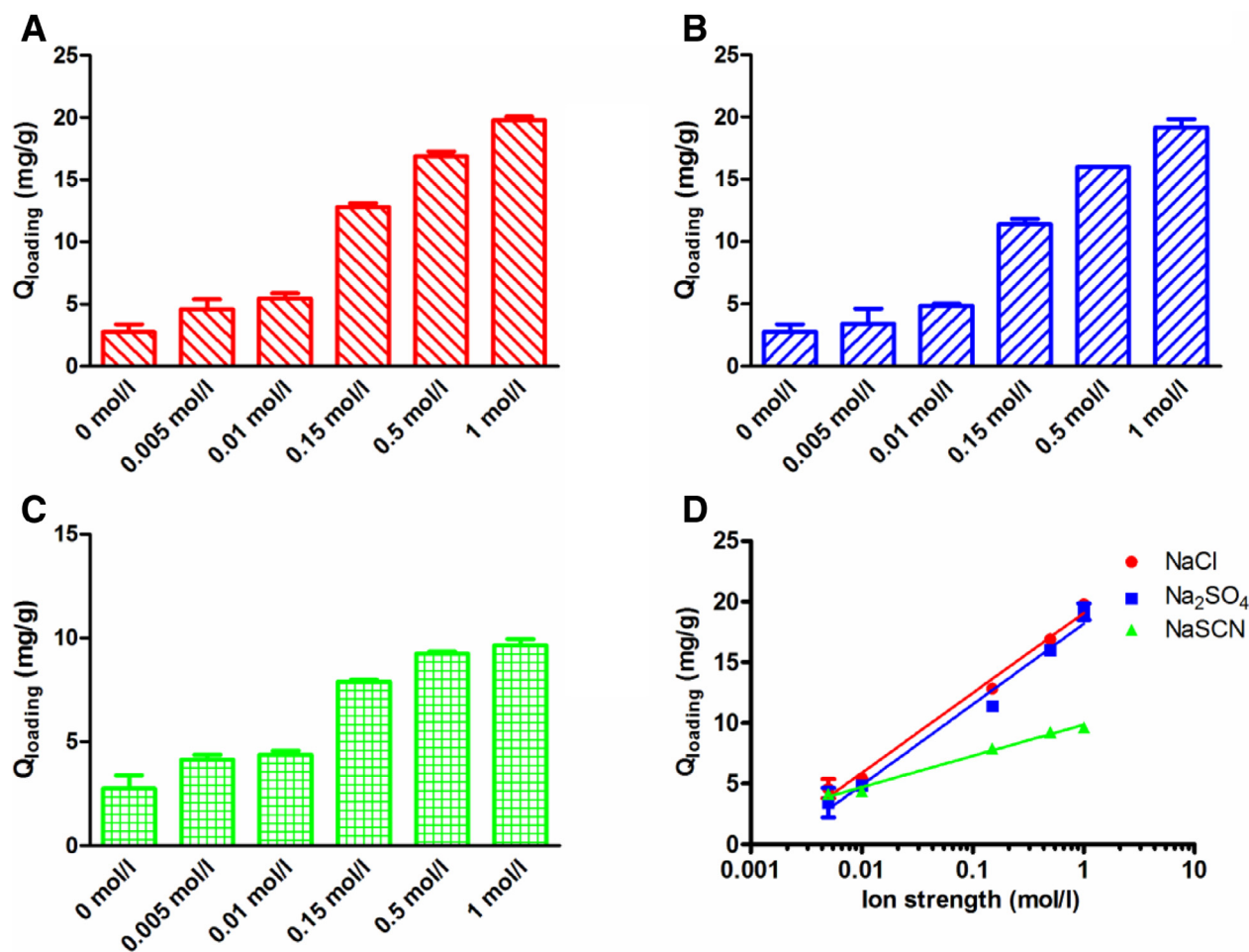
Fig. 1 – Influence of salt type on DS loading. DS loading of p-HEMA CLs from 0.5 g/l DS in salt solutions with ionic strength ranging from 0.005 to 1 mol/l using NaCl (A), Na_2SO_4(B), NaSCN(C) as adjustor and the linear correlation between the logarithms of the ionic strength and DS loading amounts of CLs in NaCl, Na_2SO_4 and NaSCN solutions (D). Each point represents the average of three measurements.

of the test at time t. The release profiles are significantly different if $f_2 < 50$.

2.8. Statistical analysis

All results were expressed as mean $\pm$ SD. Microsoft Office 2010 (Microsoft Co., Redmond, Washington State, USA) and OriginPro 8.0 (OriginLab Co., Northampton, Massachusetts, USA) were employed for statistical analysis. T-test and One-way analysis of variance were used to compare loading capacity of CLs, water content of CLs and drug solubility in different salt solutions. The differences were considered to be significant at $P < 0.05$.

3. Results and discussion

3.1. Salt effect on drug loading of contact lenses

In previous studies more attention has been paid to increase drug loading via structural modifications of CLs [17–20], the

potential influence of salts on drug loading has been ignored. Here, influence of salts concentration and salt type on drug loading in CLs was studied in detail. To eliminating the influences of solution pH on drug loading, in addition to NaCl, two other sodium salts of strong acids, Na_2SO_4 and NaSCN were used in following studies and the pH of the drug loading solutions were measured and kept constant (pH = 6.5, DS; pH = 5.5, BH; pH = 9.5, BB).

3.1.1. Influence of salt concentration
To elucidate the effect of salt concentration on drug loading of CLs, first of all, taking p-HEMA CLs as an example and DS as the model drug, influence of NaCl concentration on drug loading was investigated and the results are presented in Fig. 1A. When DS-water was used, the drug loading efficiency was only 7.58%, implying almost 92% of drug was unloaded and this was inefficient. As NaCl was added to the loading solution, except for the 0.005 and 0.01 mol/l group, all the other NaCl concentration groups enhanced DS loading significantly compared with the distilled water group ($P < 0.05$). Drug loading efficiency of 0.15 mol/l NaCl group was 37.17%. Moreover,

there existed a good linear correlation between the logarithms of the ionic strength and DS loading amount in CLs ($r = 0.9834$) (Fig. 1D), implying that it was possible to adjust the drug loading ability of CLs via changing ionic strength of the soaking solution.

3.1.2. Influence of salt type

In addition to ionic strength, whether salt type can influence drug loading in CLs at the same ionic strength needs to be clarified. Therefore, taking p-HEMA CLs as an example, the effect of salt type on DS loading in CLs was further investigated by selecting two other salts, Na_2SO_4 and NaSCN, to adjust the ionic strength in the same range of 0.005–1 mol/l, and compared with NaCl based system. Here DS concentration was kept at 0.5 g/l. As shown in Fig. 1B and C, similar to NaCl, the loading amount of DS in Na_2SO_4 and NaSCN solutions increased with increasing ionic strength but with different extent. Linear correlation also existed between logarithm of the ionic strength and DS loading in CLs in Na_2SO_4 ($r = 0.9726$) and NaSCN ($r = 0.9839$) based systems (Fig. 1D). Interestingly, it was noticed that the influence of salt type on drug loading was also ionic strength concentration dependent. When the ionic strength was under 0.01 mol/l, no remarkable influence of salt type was found, and the drug loading only increased slightly with the increase of ionic strength irrespective of salt type. However, when the ionic strength was equal or greater than 0.01 mol/l, the influence of salt type was more apparent. The effect of Na_2SO_4 and NaCl on drug loading in CLs was comparable in the ionic strength range of 0.01–1 mol/l ($P > 0.05$), but significantly lower drug loading was found in NaSCN based system compared with that of Na_2SO_4 and NaCl containing system ($P < 0.05$). Therefore, the influence of salt type is more apparent at higher ionic concentration.

3.1.3. Influence of drug type

The above study demonstrated that DS loading in CLs is dependent on salt type and concentration in the incubation medium. To clarify whether this is a general rule for drug loading in CLs, by using the same CLs (p-HEMA), the loading behavior of two other drugs with different solubility characteristics, Betaxolol Hydrochloride (BH) ($S > 1000$ g/l), and Betaxolol Base (BB) ($S = 0.67$ g/l), was studied in detail. As shown in Fig. 2A, when BH was used as the model drug, the loading amount in CLs enhanced significantly compared with the loading in distilled water irrespective of salt type ($P < 0.05$), but the extent of drug loading increase was salt type and ionic strength dependent. At physiological ionic strength of 0.15 mol/l, BH loading increased in the order NaSCN > NaCl > Na_2SO_4. BH loading in NaSCN based system was two times higher than that of NaCl based system, and three times higher than that of Na_2SO_4 based system. BH loading decreased with the increase of ionic strength in NaCl ($r = 0.8886$) or Na_2SO_4 ($r = 0.9330$) based system. In NaSCN based system, initially BH loading increased remarkably with the increase of ionic strength, but when NaSCN concentration was above 0.15 mol/l, BH loading decreased with further increase of NaSCN concentration.

As shown in Fig. 2B, when BB was used as a model drug, different phenomenon was observed. When the ionic concentration is under 0.15 mol/l, no improvement in drug loading was
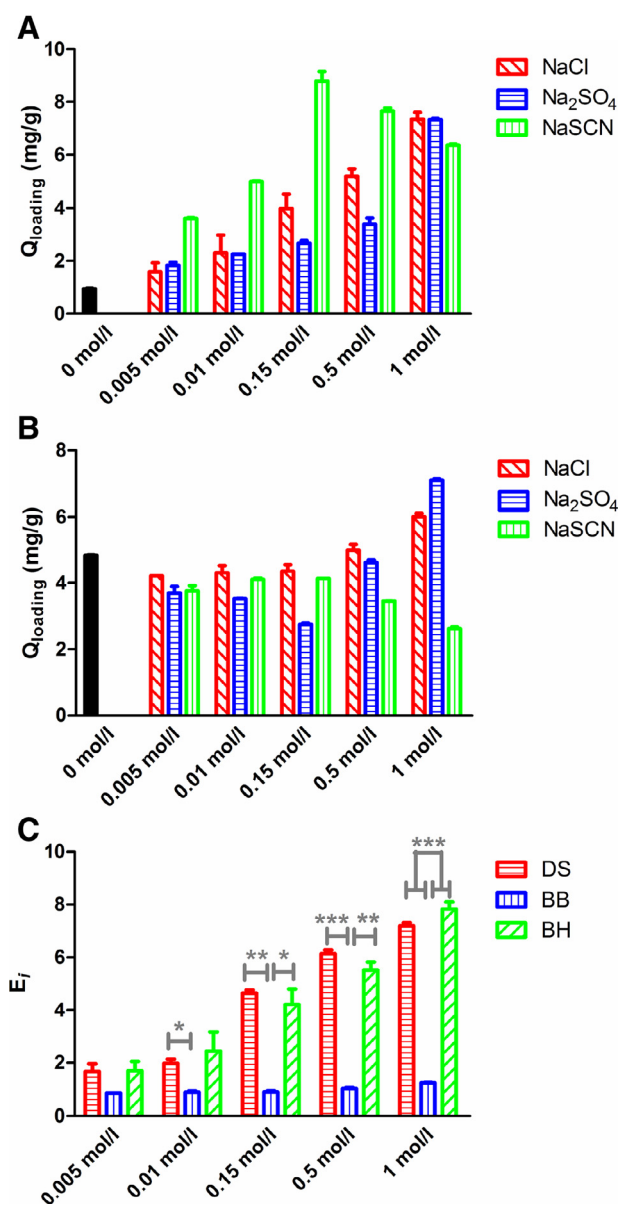
Fig. 2 – Loading results of BH (A) and BB (B) in NaCl, Na_2SO_4 and NaSCN solutions with the ionic strength ranging from 0 to 1 mol/l and comparison of enhancement factor for DS, BB and BH in NaCl solutions (C). Each point represents the average of three measurements. (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

found irrespective of salt type. When the ionic concentration was increased to 1 mol/l, significant increase in drug loading was observed in NaCl and Na_2SO_4 based system ($P < 0.05$), but drug loading decreased statistically in NaSCN based system ($P < 0.05$).

To further elucidate the influence of drug properties, taking NaCl based system as an example, enhancement factor, E_i value was calculated at different salt concentrations. As shown in Fig. 2C, E_i values are comparable for DS and BH in all the concentration ranges investigated and all the values are above one. In contrast, influence of ionic

Table 1 – Results of the fitting of the Linear and Freundlich models with the loading of DS, BH, and BB.

Drug	Solvent	Linear		Freundlich		
		K	r	n	K_F	r
DS	Water	6.77	0.9218	0.68	5.56	0.9755
	0.15 mol/l NaCl	27.77	0.9652	1.23	28.25	0.9975
BH	Water	2.79	0.8581	0.69	1.56	0.8993
	0.15 mol/l NaCl	5.83	0.9399	1.30	7.72	0.9898
BB	Water	12.89	0.8123	0.61	25.59	0.8913
	0.15 mol/l NaCl	13.25	0.8925	0.72	43.35	0.9415

concentration on the loading of BB was marginal. This study indicated that the extent of enhanced drug loading via salt addition is drug property dependent. It seems that this strategy is more effective for drugs with higher solubility (BH, S > 1000 g/l; DS, S = 17 g/l) than that with lower ones (BB, S = 0.67 g/l).

### 3.2. Mechanisms of enhanced drug loading in contact lenses

The above studies demonstrated that salt concentration, salt type and properties of drug can impact drug loading in p-HEMA contact lenses. However, the mechanism behind is unclear and needs to be explored.

3.2.1. Influence of salts on absorption isotherms

Absorption isotherms are commonly used to describe the distribution of drug between CLs and solutions in the equilibrium state. By using distilled water and 0.15 mol/l NaCl as the incubation solvent, the absorption isotherms of three model drugs in p-HEMA based CLs were studied and fitted into two models, linear and Freundlich absorption isotherms, the results are presented in Fig. 3 and Table 1. It was found that no matter distilled water or 0.15 mol/l NaCl was used as solvent, Freundlich isotherm got the best fit with experimental data for all the drugs, implying that the nature of CLs drug loading might be drug absorption on three-dimensional network of the lens [25] and salt addition has no influence on drug absorption mechanism. In the case of DS and BH, K_F, the Freundlich isotherm constants increased appro. 5 fold in 0.15 mol/l NaCl based solution compared to that in distilled water, implying a higher absorption capacity [26]. Similarly, all the values of Freundlich exponent factor in salt groups ($n > 1$) were higher than that in water groups ($n < 1$), indicating more favorable condition for drug absorption in salt containing solutions [27]. When BB was used as the model drug, drug absorption behavior in both distilled water and 0.15 mol/l NaCl based solution was comparable, no remarkable influence of salt on drug absorption extent was disclosed.
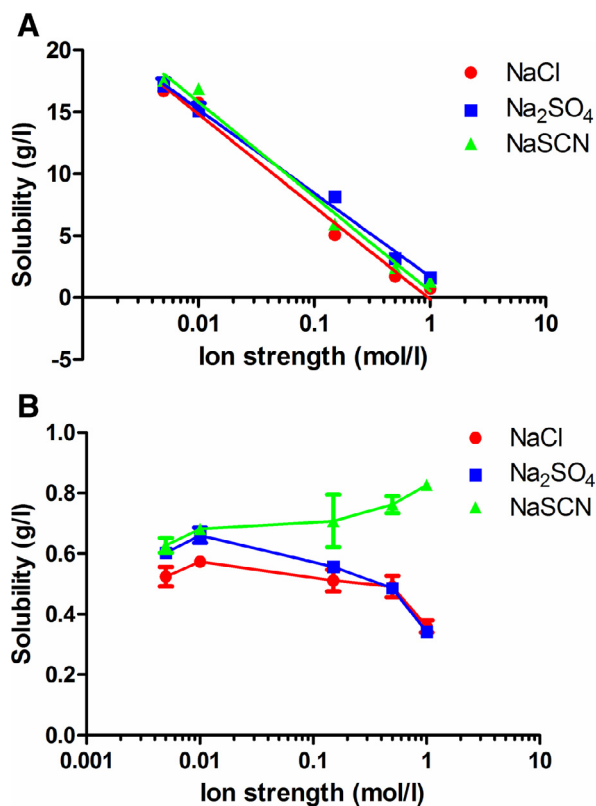
3.2.2. Influence of salts on drug solubility

The absorption isotherms studies indicated that the nature of drug loading might be absorption on CLs irrespective of ions concentration in the incubation solution. It is well known that
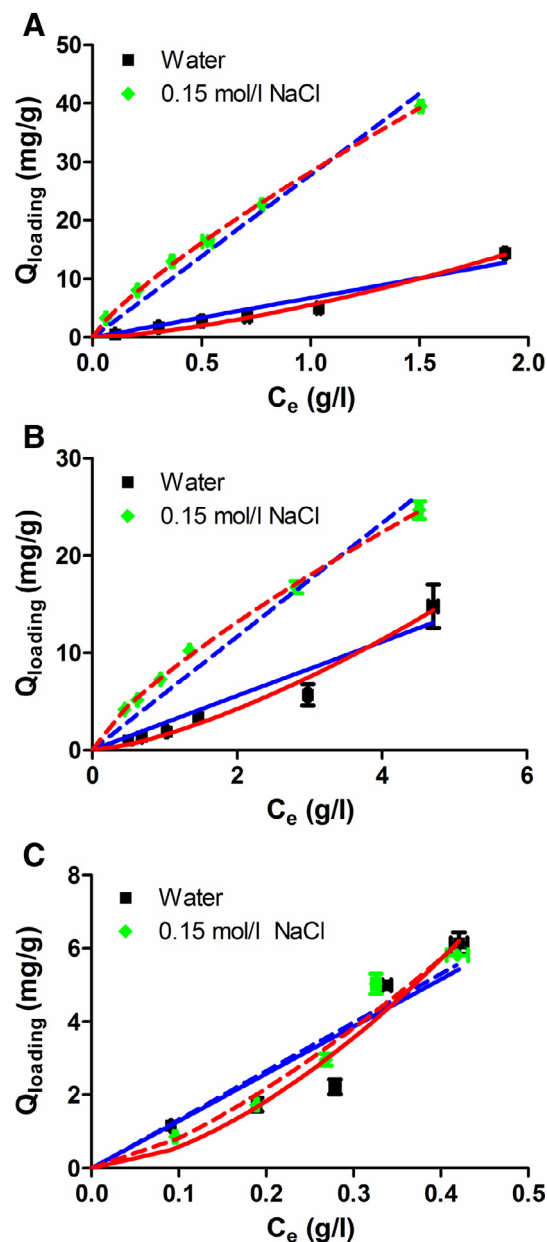
Fig. 3 – Absorption isotherms of DS (A), BH (B), BB (C) in water (square and solid line) or 0.15 mol/l NaCl (diamond and dash line). Fitting curves were obtained based on the two models, linear-type model (blue) and Freundlich-type model (red). Each point represents the average of three measurements. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the absorption in aqueous solution is a complex process involving the intermolecular action between solute and solvent, solute and adsorbent, adsorbent and solvent. Drug solubility could be used as an indicator of solute–solvent affinity. It is assumed that salt addition may increase solute–adsorbent affinity by decreasing solute–solvent affinity, therefore leading to decreased drug solubility and enhanced drug loading.

Fig. 4 – Influence of salt on apparent solubility of drugs. DS (A) and BB (B) solubility in NaCl, Na₂SO₄ and NaSCN solutions with the ionic strength ranging from 0.005 to 1 mol/l. Each point represents the average of three measurements at 25 °C.

To test this hypothesis, influence of salt addition on drug solubility was investigated. Since the solubility of BH in water is extremely high (>1000 g/l), which is difficult to accurately quantify, only the solubility of DS and BB in different salt solutions was measured. As shown in Fig. 4A, when DS was used as the model drug, irrespective of salt type, drug solubility decreased significantly with the increase of ionic strength. A linear correlation existed between the logarithms of the ionic strength and DS solubility ($r = 0.9880$, NaCl; $r = 0.9899$, Na₂SO₄; $r = 0.9874$, NaSCN). This was in good agreement with the influence of ion concentration on drug loading in CLs, as presented in Fig. 1B. The decrease of DS solubility by salt addition might be explained by the common ion effect. For DS, which was a salt form of diclofenac, the solubility product could be described by:

$$D-Na(S) \rightleftharpoons D^- + Na^+ \tag{12}$$

$$K^\circ_{sp} = a_{D^-} \cdot a_{Na^+} = D^- \cdot Na^+ \cdot (\gamma_\pm)^2 \tag{13}$$

where 'a' is the activity, $\gamma_\pm$ is the mean ionic activity coefficient, D^- and Na^+ are the concentrations of the cation and the anion, respectively. Adding NaCl/Na₂SO₄/NaSCN to the solution would increase the concentration of the cation, Na^+ and in order to keep the K°_{sp} constant, the final result was to de-

Table 2 – Setschenow constants for Diclofenac Sodium and Betaxolol Base in different salt solutions with the ionic strength ranging from 0.005 to 1 mol/l.

| Salt type	Setschenow constant	
	Diclofenac Sodium	Betaxolol Base
NaCl	1.37 ± 0.13[a]	0.19 ± 0.02
Na₂SO₄	1.03 ± 0.08	0.25 ± 0.01
NaSCN	1.14 ± 0.12	-0.12 ± 0.02
[a] Standard deviation $n = 3$.		

press the drug's aqueous solubility. The Setschenow constants calculation, as presented in Table 2, further demonstrated that salting out is the main reason for decreased solubility of DS by salt addition.

When BB was used as the model drug, apparent influence on drug solubility was observed only when the ionic concentration was above 0.15 mol/l (Fig. 4B). Above this point, NaCl and Na₂SO₄ addition caused salting-out effect, with the solubility of BB decreased significantly ($P < 0.05$), in contrast, NaSCN led to salting-in effect, with statistical increase in drug solubility ($P < 0.05$), as demonstrated by the calculated Setschenow constants (Table 2), with negative value in NaSCN added system. These data are in good agreement with the influence of ion concentration on drug loading in CLs, as presented in Fig. 2B, with decreased solubility corresponding to increased drug loading in CLs.

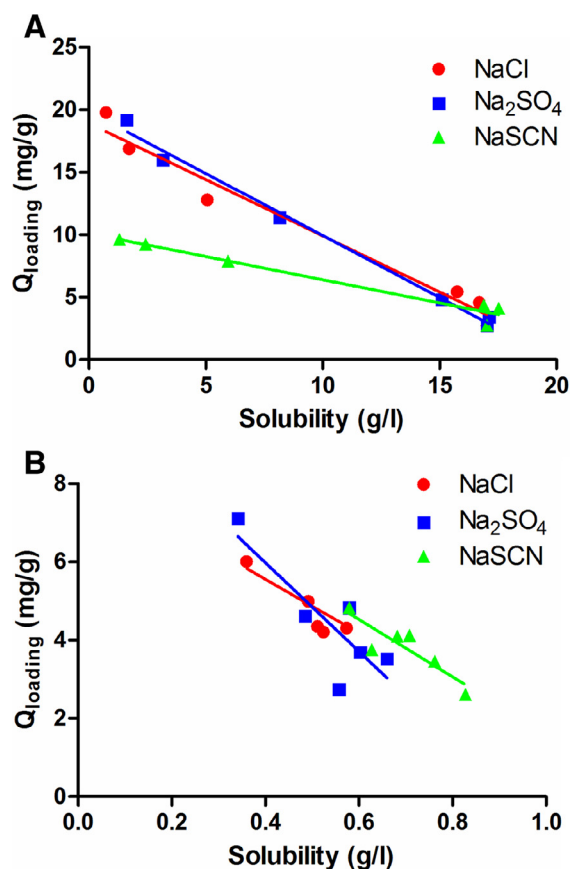*3.2.3. Influence of salts on water content of contact lenses*
Water content is an important parameter related to eye comfort. Previous studies show that drug loading in soft CLs mainly depends on the water content and interaction between drugs with polymer components [28]. For drugs without interaction with CLs, high content of water in CLs would dissolve more drugs and then a high drug loading could be achieved [29]. In addition to drug solubility change, to test whether water content variation in CLs contributed to drug loading difference when salt solutions were used, and to get a deep understanding of the influence of salts on the water content of CLs, both total water and bound water of contact lens were measured using salt solution as solvent.

First of all, to elucidate the effect of salt concentration on water content of CLs, influence of NaCl concentration on p-HEMA CLs water content was investigated and the results are presented in Fig. 5A. When NaCl concentration was 0.005 or 0.15 mol/l, no statistical differences in both total water content and bound water content were found compared with the distilled water group ($P > 0.05$), thereafter, further increasing NaCl concentration to 1 mol/l caused significant decrease of total water content ($P < 0.05$), with free water almost completely removed. As for bound water, it decreased from 55.3% in 0.15 mol/l NaCl group to 42.5% in 1 mol/l NaCl group ($P < 0.01$). Those data demonstrated that the total and bound water content in contact lens could be adjusted via changing ionic strength of the immersion solution. Similar trend was observed with Na₂SO₄ containing system (Fig. 5B), with water content unchanged under lower or physiological ion conditions (0.15 mol/l), and significantly decreased water
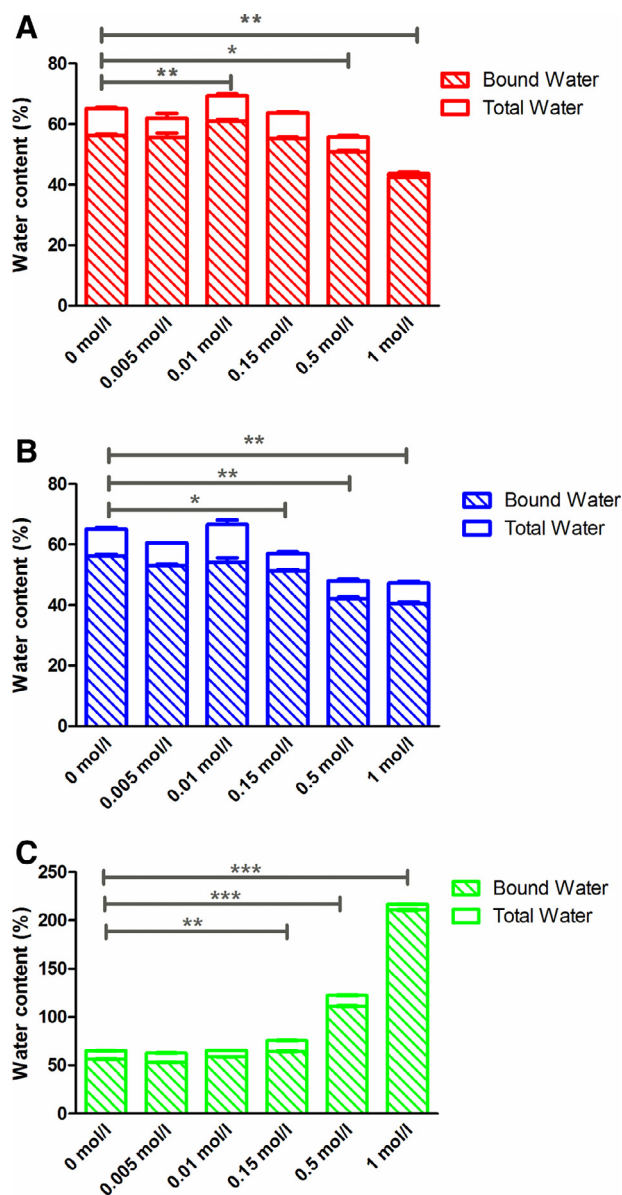
Fig. 5 – Water content of p-HEMA contact lenses in NaCl (A), Na_2SO_4 (B) and NaSCN (C) solutions with the ionic strength ranging from 0.005 to 1 mol/l. Each data point represents the average of three measurements and all the statistical tests are based on the data of bound water content. (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

Fig. 6 – Relationship between drug loading amount of CLs with apparent aqueous solubility of DS (A), BB (B) in NaCl, Na_2SO_4 and NaSCN. Each data point represents the average of three measurements.

content at higher ion concentration, implying increased drug loading at lower or physiological ion conditions was not related to the water content in the CLs. In contrast, a reverse case was observed at higher ionic concentration when NaSCN was used as the salt for ionic strength adjustment, with the bound water content increased to 210.7% at 1 mol/l NaSCN compared to 64.3% at 0.15 mol/l (Fig. 5C). The various effects of salt type on water content of CLs might be explained by the structure-maker or structure-breaker ability of salts used. Kosmotropes agents, NaCl and Na_2SO_4, would bind water molecules strongly and increase water structure, and then less water would have the opportunity to be adsorbed on poly-

mer matrix at higher ionic strength. However, for chaotropes agents, NaSCN, the opposite phenomenon would occur [23].

3.2.4. Relationship among drug solubility and water content with drug loading of contact lenses

The above study demonstrated that salt addition can influence drug solubility in solution, and at higher ionic concentration, bound water in CLs can also be influenced. Then, what is the relationship among drug solubility, water content of CLs and drug loading in CLs?

To answer this question, first of all, the relationship between drug solubility and drug loading in CLs was characterized. As shown in Fig. 6A, a good negative correlation between DS loading in CLs and DS solubility in different soaking solutions was established irrespectively of salt type ($r = 0.9678$, NaCl; $r = 0.9820$, Na_2SO_4; $r = 0.9486$, NaSCN). Similar trend was observed when BB was used as the model drug (Fig. 6B).

The negative relationship between drug loading in CLs with its apparent aqueous solubility indicated that drug solubility might be an important factor determining drug loading in CLs. However, when the ionic strength was greater than or equal to 0.15 mol/l DS solubility in NaCl was statistically lower than that in Na_2SO_4 (Fig. 7C), but no difference in DS loading between the two salt groups was found (Fig. 7A). It implied that drug solubility might not be the only factor determining
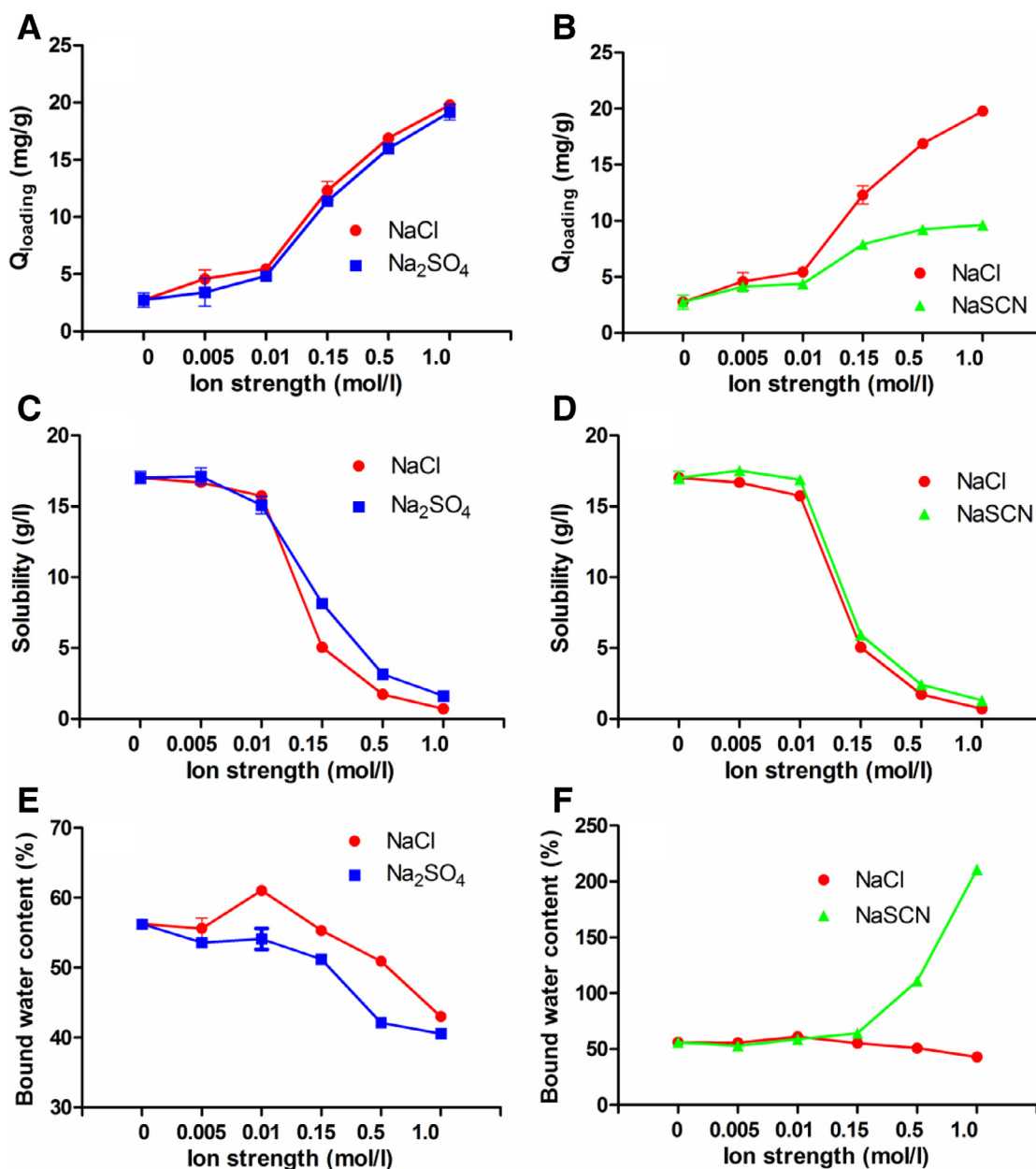
Fig. 7 – The variations of DS loading of p-HEMA CLs (A and B), solubility (C and D) and bound water content (E and F) as a function of ionic strength using different salts. Each data point represents the average of three measurements.

drug loading in CLs, especially at high ionic concentration. As shown in Fig. 7E, higher bound water content was found in NaCl based system compared with Na_2SO_4 based system with the increase of ionic strength. Due to the immobilization of bound water, it may be difficult to dissolve and transfer drug but might decrease drug loading [30,31]. Since free water content in CLs is quite low, its influence on drug loading could be ignored. Therefore, the comparable DS loading in NaCl and Na_2SO_4 based system might be a compromise between the influence of drug solubility and bound water content in the CLs (Fig. 7E). Similar phenomenon was observed when NaSCN based system was compared with NaCl containing system, as presented in Fig. 7B, D and F.

Based on the results and discussion presented above, the possible mechanism of salts effect on drug loading of CLs is schematically presented in Fig. 8. The change of ionic strength in soaking solution might affect drug loading by affecting drug apparent solubility and altering bound water amount. The apparent solubility can be used as a parameter to reflect the affinity between drug and solvent, the higher the solubility, the better the affinity. Similarly, content of bound water can be used to represent the affinity between CLs and solvents. A better affinity will lead to higher water contents. If when salts added, both the apparent solubility of drug and the bound water content of CLs decrease, it indicates the decrease of drug-solvent and lens-solvent affinity,
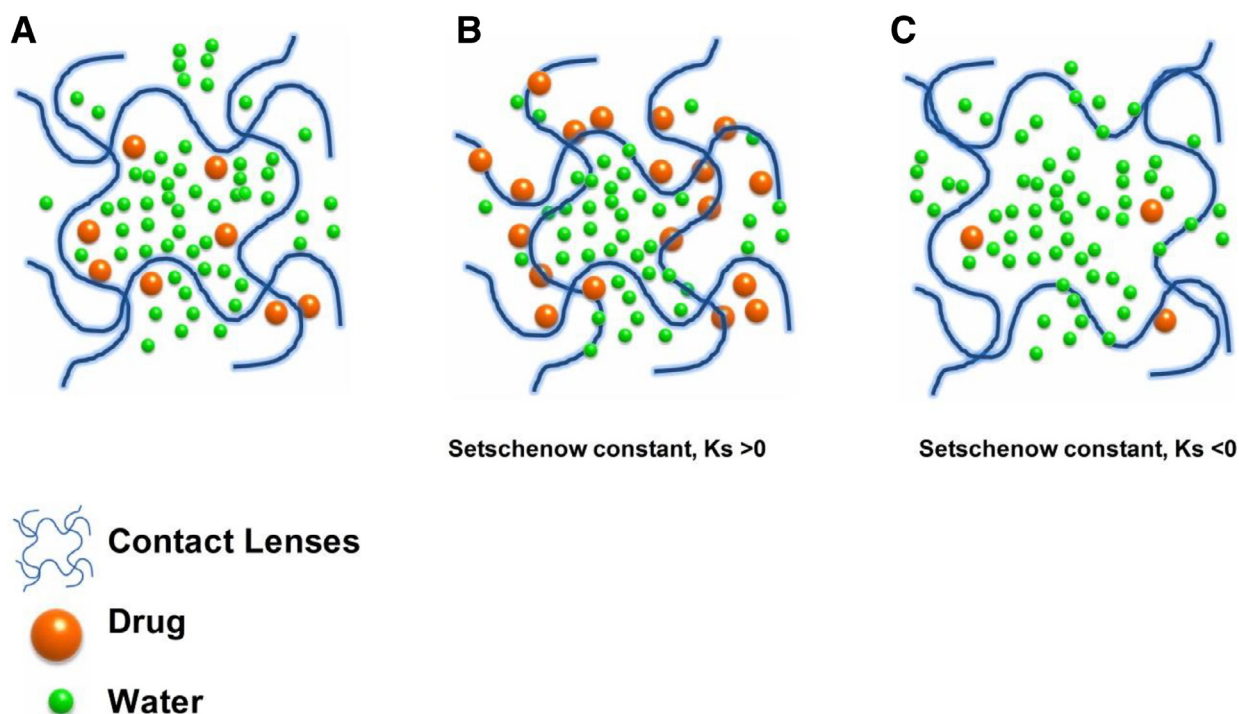
Fig. 8 – Mechanisms of drug loading modulation in CLs by adjusting the ionic strength of leading solutions. (A) drug loading in water; (B) loading enhancement caused by salt; (C) loading decrease caused by salt.

which will enhance drug loading in CLs synergistically, liking a combination of "squeezing out" and "leaving space" process, resulting in higher drug loading (Fig. 8B). An opposite situation will happen if salt addition causes drug solubility and bound water content increase, leading to drug loading decrease (Fig. 8C).

3.3. Feasibility of improving CLs drug loading by adjusting the ionic strength of loading solutions

To further investigate whether this simple method is also applicable when crosslinker amount or the compositions of CLs are changed, pHEMA CLs with different degree of crosslinking (0.1%; 0.5%; 1%; 2%, w/w), pHEMA CLs with various functional monomers (VP or MAA, 2%, w/w), silione-hydrogel CLs (HEMA/NVP/TRIS, 20/40/40, w/w/w) were prepared and DS loading via immersing CLs in 0.5 g/l DS-water or 0.5 g/l DS-0.15 mol/lNaCl solution was investigated. As shown in Fig. 9A according to the value of E_i, adjusting ionic strength of the loading solution improved drug loading significantly compared with distilled water based system ($P < 0.05$), but the extent of E_i value increase was CL property dependent. Although degree of cross linking had an influence on drug loading, there was no definite dependence of drug loading on crosslink density and this was in agreement with previous reports [18,19].

In any case, the light transmittance of CLs should not be influenced by the drug loading process. To test the potential influence of salt-enhanced drug loading on the application of CLs, the light transmittances of HEMA based CLs were tested after immersed in water, 0.5 g/l DS, 0.5 g/l DS-0.15 mol/l NaCl, and 0.5 g/l DS-1 mol/l NaCl for 3 d (Fig. 9B), no apparent change in light transmittance was observed after salt addition. Light transmittance of p-HEMA CLs were above 95% at 500 nm in all cases.

To figure out whether changing ionic strength of loading solution had an influence on drug release profile, DS release from CLs prepared with 0.5 g/l DS solution using water, 0.15 mol/l or 1 mol/l NaCl as solvents was investigated in STF and the results are shown in Fig. 9C. Almost 80% of DS was released in the first 2 h and this is in agreement with previous reports [29]. Slightly higher drug release was found at time points 0.5 h and 1 h for 0.15 mol/l NaCl based system, but no statistical difference between the release profiles of distilled water and 0.15 mol/l NaCl based systems was found ($f_2 = 52.61$). Similarly, DS release from 1 mol/l NaCl based system was comparable with that from water based system ($f_2 = 62.38$).

4. Conclusions

In this paper, it was found that adjusting the ionic strength of loading solutions resulted in significant increase of drug loading in CLs. Type and concentration of the salts, solubility of the drug were the main factors influencing drug loading. The mechanism for improved loading was likely related to the reduced drug solubility in loading solutions and the reduced bound water content in contact lenses. Modulation of drug loading by adjusting ionic strength was also
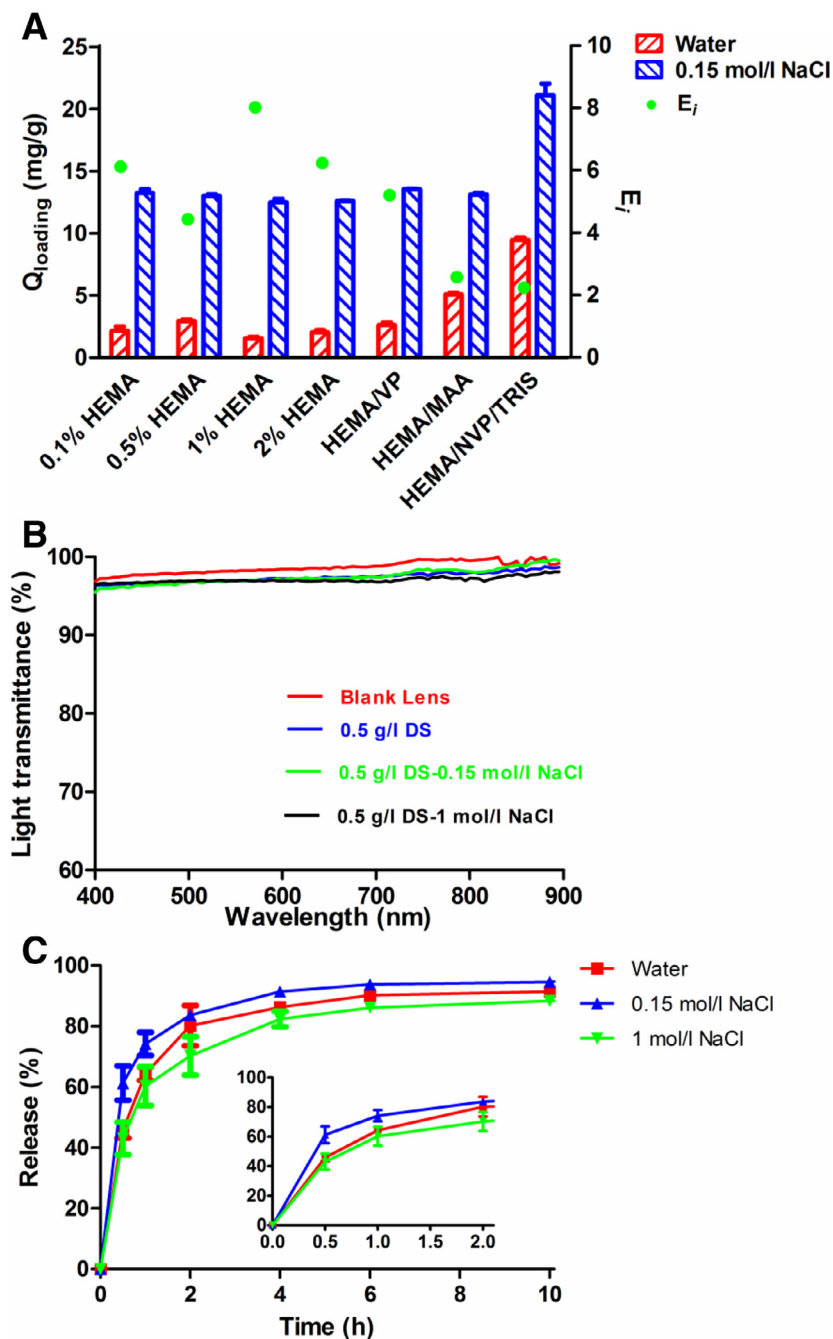
Fig. 9 – DS loading amount (left Y) and E_i (right Y) of 0.15 mol/l NaCl using different composition contact lenses (A); Light transmittances of p-HEMA contact lenses using water; 0.5 g/l DS; 0.5 g/l DS-0.15 mol/l NaCl or 0.5 g/l DS-1 mol/l NaCl as immersion solution, respectively (B); DS release from CLs loaded with 0.5 g/l DS in water, 0.15 mol/l NaCl or 1 mol/l NaCl separately using STF as release medium. The inserting graphs represent drug release in 2 h (C).

applicable to other CLs and the light transmittance was not affected. This method was more suitable for salt-form drugs with high solubility. Since drugs in salt form account for a considerable proportion in ophthalmic drugs on the market, a broad application of this method can be expected and further explored. In summary, it was demonstrated that adjusting ionic strength of loading solution may be an economical and effective way to improve drug loading in CLs. This simple method can also be useful for other drug delivery systems based on hydrogel. The number of drugs used in this study was quite limited and more drugs should be used to verify the feasibility of this method in the near future.

Conflicts of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

Acknowledgments

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ajps.2018.05.002.

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