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Elucidation of Colloid Performances of Thermosensitive *In Situ*—Forming Ophthalmic Gel Formed by Poloxamer 407 for Loading Drugs

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ABSTRACT

The herein work elucidated colloid performances of thermosensitive *in situ*—forming ophthalmic gel established by poloxamer 407 (F127), mainly including facile and simple methods (load-bearing method, tilting plate method, and eye simulation method) to analyze and evaluate F127 gel and intensively addressed capacity of F127 gel for loading drugs. The results demonstrated that the poorly water-soluble berberine hydrochloride improved entangled intensity of F127 and therefore reduced the sol dynamic rate, which contributed to lower gelation temperature and enhancement of cornea adhesion, further lowering the rate of gel corrosion together with drug release. Importantly, the addition of hydroxy propyl methyl cellulose (HPMC) K15M contributed to stronger gel strength because strong interaction forces were formed between HPMC K15M and F127 when the molecular chain segments of HPMC K15M interspersed among F127 network. All the aforementioned results provide valuable instruction for designing and evaluating thermosensitive *in situ*—forming ophthalmic gel.

Introduction

It is known that gel belongs to one type of colloid systems. Ophthalmic gel has long been studied to solve the problem that unique anatomy and physiology of the eye make it difficult for drug delivery into desired eye tissues.[1] Liquid ophthalmic formulations show low bioavailability because of constant drainage in the eye. Some polymers can be used to prepare eye drops, including cyclodextrin.[2] The normal drainage of an instilled drug dose commences immediately on instillation and is almost completed within 5 min.[3] Furthermore, the main part of the administered drug that is transported by tear drainage may cause side effects.[4] In recent years, many pharmaceutical scientists have focused on developing *in situ* gel-forming systems to overcome the poor bioavailability and therapeutic response exhibited by conventional ophthalmic solutions.[5] *In situ* gels are designed as polymer solutions which can be administrated as liquid and undergo a phase transition to semisolid gel on exposure to physiological environments. The gelation lowered the rate of diffusion and erosion of both the polymer and the entrapped drug, thereby enhancing the drug retention and bioavailability.[6] On the other hand, *in situ*—forming gel should be able to turn into *in situ*—forming sol after diluting with tears, thus avoiding the block of the lacrimal duct. In general, the gelation of a polymeric solution can be triggered by a number of factors,[7] including pH (cellulose acetophthalate[8] and carbopol[9]), temperature (ethyl/hydroxyethyl cellulose,[10] poloxamers,[11] and chitosan[12]), the presence of cations (alginate[3]), or light.[13] Among them, a promising strategy appears to be gelation triggered by a temperature change because platforms' properties can be easily tuned as a function of therapeutic needs and administration routes.[14] Nowadays, thermosensitive *in situ*—forming gel has been widely investigated for ophthalmic applications.[15,16] However, simple methods to analyze and evaluate thermosensitive *in situ*—forming ophthalmic gel are not well documented.

One of the well-known polymer types possessing thermoresponsive behavior is pluronics, which is named as poloxamers. Poloxamer 407 (F127) belongs to a family of more than 30 ABA block copolymers, in which a hydrophobic polypropyleneoxide (PPO) block is sandwiched between 2 hydrophilic polyethyleneoxide (PEO) blocks. In general, poloxamers behave like nonionic surfactants attributing to the amphiphilic nature of their block units. These polymers form thermoreversible gels in concentrated aqueous solutions, and the higher the concentration, the stronger the gel strength.[17] Poloxamers have been widely used as an ocular

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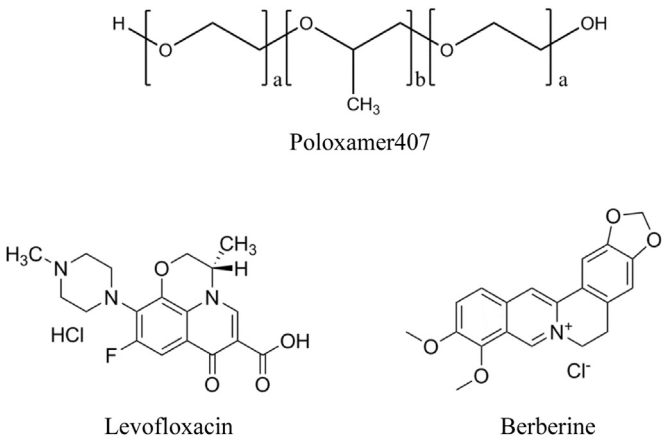


Figure 1. Chemical structure of F127, LOF, and Ber.

drug delivery system because they could prolong drug release and present satisfactory inertia for eye tissue. The brief introduction of 2 model drugs is provided as follows. Water-soluble levofloxacin hydrochloride (LOF) with characteristics of wide antimicrobial spectrum and strong antibacterial effect kills bacteria by inhibiting the activity of bacterial DNA gyrase.[18] The ophthalmic gel product of LOF has been listed and is applicable for bacterial conjunctivitis, corneal ulcer, and postoperative off-grade eye infection. Poorly water-soluble berberine hydrochloride (Ber), a derivative alkaloid, widely exists in medicinal herbal plants (*Phellodendron chinense* Schneid, Mahonia, Fake porcupine thorns, *Berberis vulgaris*, *Coptis chinensis*, *Berberis aquifolium*, etc.) with a variety of pharmacological effects such as antitumor activity, antibiotic property, antioxidant and anti-inflammatory effects and is useful in the treatment of gastroenteric discomfort and diabetes in clinic.[19]

In the current stage, the *in situ*—forming ophthalmic gel of Ber aiming to cure ocular inflammation is still under study. Hydroxy propyl methyl cellulose (HPMC) is one of the principal cellulose derivatives and has been used in a broad range of applications in pharmaceutical and food formulations. HPMC is a nonionic cellulose derivative with methyl (hydrophobic) and hydroxypropyl (hydrophilic) groups added to the anhydroglucose backbone and include a family of cellulose ethers that differ principally in molecular weight, viscosity, and degree of substitution.[20] Usually, HPMC can be added as an auxiliary material in gel formulation with potential ability to improve gel strength. Chitosan can also be considered as an assistant for poloxamer gels owing to its biocompatibility, biodegradability, and capacity to improve the mucoadhesiveness and gel strength when enough concentration is reached.[21] To develop and standardize thermosensitive *in situ*—forming ophthalmic gel, it is necessary to investigate gelation process and a series of parameters, mainly including sol dynamics, gelation temperature, and biological adhesion. The present study describes facile and simple methods (load-bearing method, tilting plate method, and eye simulation method) to analyze and evaluate F127 in constructing thermosensitive *in situ*—forming ophthalmic gel. (The chemical structure of polymer and drug are shown in Fig. 1.) Apart from these facilely designed methods, novel studies of this article include (1) the logical analysis of the sol-to-gel process for either blank gel excipient or drug-loaded gel; (2) drug impacts on formation and application of thermosensitive *in situ*—forming gel; (3) how extra HPMC favors the application of thermosensitive *in situ*—forming gel. It is sure that confidential that the whole work provides wonderful instruction for the design and application of thermosensitive *in situ*—forming ophthalmic gel.

Materials and Methods

Materials

F127 and HPMC K15M were purchased from the BASF company (Germany). The model drugs (LOF and Ber) were bought from Melone Pharmaceutical Co. Ltd. (Dalian, China).

Preparation of Blank Sol and Drug-Loaded Sol

Briefly, blank sol was obtained by completely dissolving F127 in cold deionized water (18%, w/w) and stored at 4°C refrigerator overnight. The preparation of drug-loaded sol also applied the cold method except that 15 mg of drug was added into 5 g of F127 solution until well distributed.

Sol Dynamics

The tilting plate method was conducted as follows. The glass container was kept at a tilt angle of 45° at room temperature (25°C) for 10 min. Then, 40 μL sol samples (blank sol, LOF-loaded sol, and Ber-loaded sol) were poured dropwise onto a glass plate, and the time taken for each drop to slip down for a certain length was noted. The sol kinetic curve was obtained by drawing displacement-time curve according to these data.

The Observation During Sol to Gel

To vividly describe the morphologic changes from sol to gel and present the impacts of drug on gel formation, optical microscopy (ANTI-Mould Nikon, China) was used to observe blank gel and drug-loaded gel.

Measurement of Gelation Temperature

The load-bearing method was applied to study gelation temperature of blank gel and drug-loaded gel. All the samples were stored at 4°C until further use. Samples were poured into vials remaining in water bath at high temperature (50°C), and gels formed gradually. Then, a small steel ball with quite a small weight (dimension $\leq$3 mm) after being preheated was placed on the surface of gels gently. The surrounding temperature of gels was lowered down slowly, and the gel strength lowered with decreasing temperature. The steel ball would fall down when gel strength was not strong enough to hold it. The temperature at the moment the steel ball fell down, which was the gelation temperature of this sample, was noted down. The mean of 3 replicates was taken for calculation.

Cornea Adhesion

The contact time of drug on the surface of cornea is the primary determinant factor for the ophthalmic preparation efficacy. Under the condition of good compliance of patients, the extension of residence time on eyes can improve the bioavailability of drug and reduce systemic side effects and drug use frequency. The egg shell membrane, which is also called the shell, is the organic fiber made of tough keratin. The cornea is a horizontal oval transparent biological film, and its main component is protein. Owing to the similar shape and composition between the eggshell membrane and the cornea, we chose the eggshell membrane to mimic cornea with the aim to study corneal biological adhesion of blank gel and drug-loaded gel.

Initial work was carried out to test the corneal adhesion of blank sol and drug-loaded sol. The fresh egg shell membrane was spun out from eggs and carefully spread onto the smooth surface of an

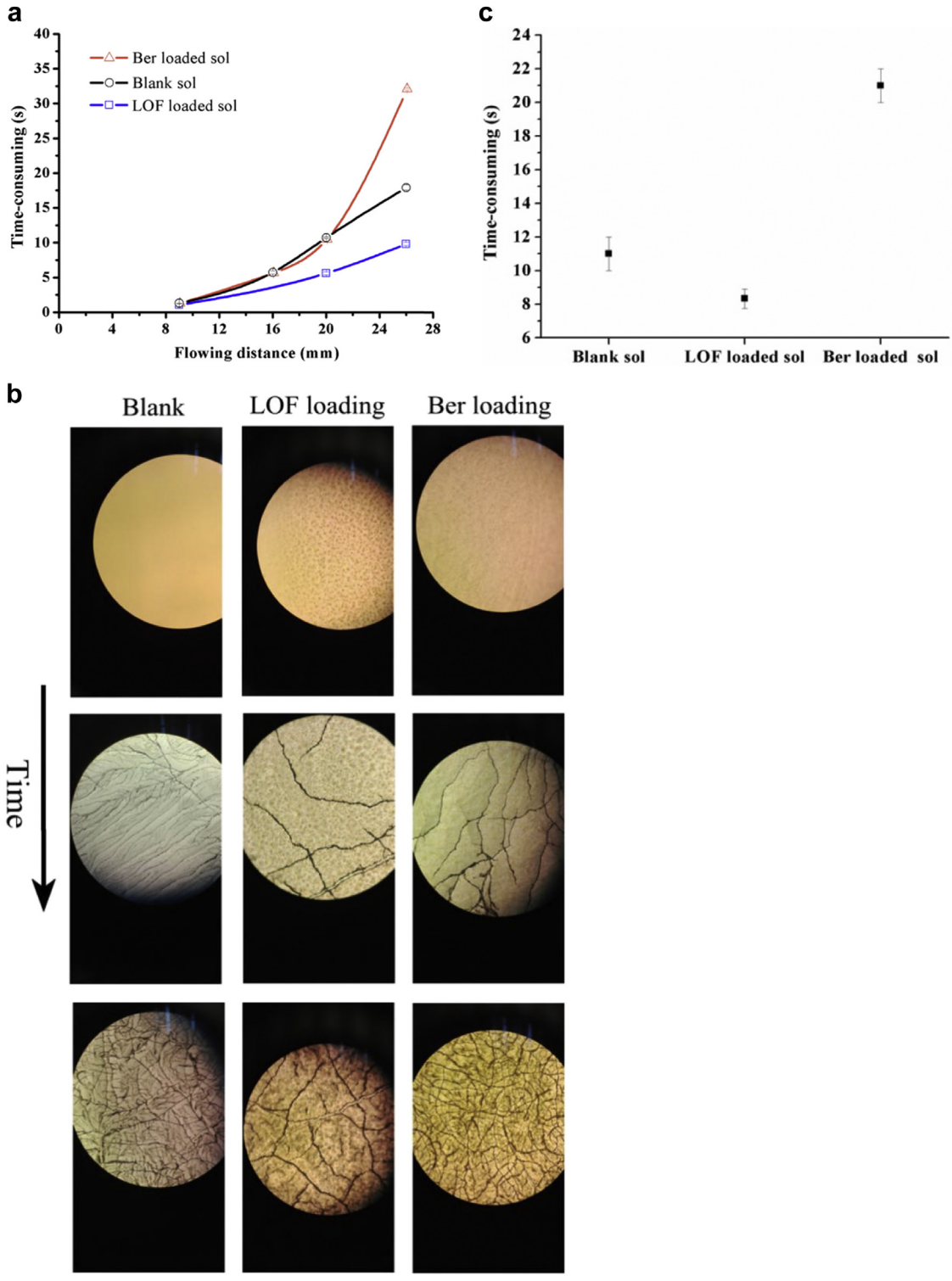


Figure 2. Sol dynamics result of blank sol, LOF-loaded sol, and Ber-loaded sol.

ellipsoid container at room temperature (25°C). Sol sample (40 μL) was poured dropwise onto the eyelid fornices and the time was noted. The time for these sol samples to flow the same distance was noted down.

In the following section, corneal biological adhesion of blank gel and drug-loaded gel was studied by using the elution method, which can reflect the residence time of gels on the eye. Briefly, the fresh egg shell membrane was spread onto the smooth surface of the ellipsoid container, and the container was kept in the water bath at 37°C. To simulate the eye environment, the fresh egg shell membrane was immersed in the water bath while only the top part was exposed. A liquid of sol sample (40 μL) was poured dropwise onto the eyelid fornices, and the time was noted. It was clear that the sol quickly converted to *in situ* gel at the moment it contacted onto the eye. The eye was washed using 35°C simulated tear liquid with the speed of 40 μL/min until complete corrosion of gel sample.

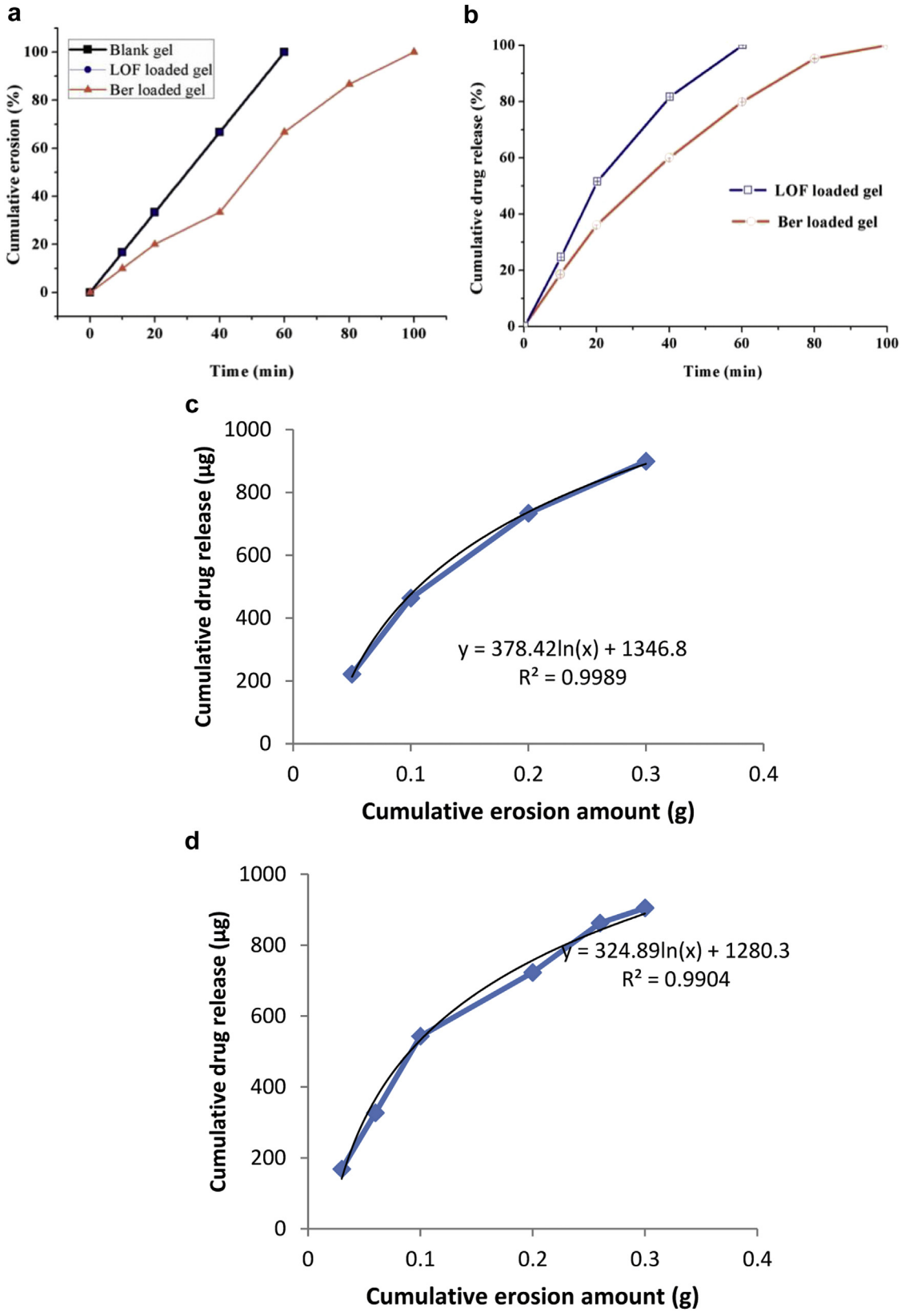


Figure 3. (a) Optical images of samples (F127, LOF loading, Ber loading) from sol to gel; (b) cornea adhesion study of blank sol, LOF-loaded sol, and Ber-loaded sol; (c) gel corrosion and drug release of LOF-loaded sol (a); Ber-loaded sol (b); fitting curves of LOF-loaded sol (c); and Ber-loaded sol (d).

Gel Corrosion and Drug Delivery

Blank sol and drug-loaded sol (0.4 mL) was precisely measured into the weighed EP tube and kept in a water bath oscillator balance at a constant temperature (35°C). The sol quickly turned into gel in the EP tube, and the weight was recorded. Afterward, 5 mL preheated simulated tear liquid was added into the EP tube along the tube wall, followed by the start of the oscillator balance. All the medium in the EP tube was withdrawn at different time intervals (10 min, 20 min, 40 min, 60 min, 80 min, and 100 min), the EP tube was weighed, then the preheated 5 mL simulated tear liquid was added, and the process was continued. The decreased weight in the EP tube was the corrosion amount of gel sample, by which cumulative gel corrosion amount through time was calculated. UV spectroscopy was applied to measure the adsorption of LOF and Ber in the withdrawn medium to calculate the cumulative drug release from the drug-loaded gel. The correlation between gel corrosion and drug release was further discussed. Both cornea adhesion and gel corrosion together with drug release were analysed with the eye simulation method.

Molecular Dynamics of Drug-Loaded Gel

The structure of LOF, Ber, and F127 was drawn by ChemDraw software. The 3D structures of macromolecules F127 were created by optimization involving structural minimization and structural dynamics optimization with the Sybyl 6.9.1 software package. The composites were optimized by means of Materials Studio software. First, a box was built, and the water and the molecules that needed to be optimized were placed in the box. Second, geometry optimization was performed with COMPASS force field, and other parameters were maintained at the default values. Third, dynamics optimization was studied using the following parameters: Ensemble: NPT、Time step: 1.0 fs、Number of steps: 50,000、Energy: COMPASS. All other parameters were maintained at the default values. After optimization of the dynamics, pictures of temperature, energy, and kinetic density were obtained.

HPMC as the Auxiliary Material for F127 Sol

The high concentration of F127 is always used to prepare *in situ* gel, which ensures enough sensibility to temperature changes. HPMC can be used mainly to adjust gel strength of F127 aiming to increase the cornea adhesion and improve degree of drug release and other purposes. One end of the balance was fixed on the disc, and the disc was displayed under the 2 cm surface bottom. After the gel is formed, weight was added with a constant speed to equilibrate the other end and the weight was recorded when the disc could be dragged out to represent gel strength. Briefly, 0.1%, 0.2%, and 0.5% of HPMC K15M was, respectively, added into 18% F127 sol and stirred evenly. The gel strength of these samples was measured at 35°C. To highlight the function of HPMC, gel strength of another cellulose chitosan was also analyzed.

Molecular Dynamic of F127 Sol With HPMC or Chitosan

To further confirm the impact of HPMC and chitosan on F127 gel strength, molecular dynamic analysis was performed. The 3D structures of macromolecules (F127, HPMC, and chitosan) were created by optimization involving structural minimization and structural dynamics optimization with the Sybyl 6.9.1 software package. The parameters of optimization were energy change 0.005 (kcal/mol), max iterations 10,000, charges using the Gasteiger-Huckel method, and minimization with the Powell method (Tripos force field) to an energy change of 0.005 kcal/(mol×A). All other parameters were maintained at the default values. Molecular dynamic analysis was optimized by Materials Studio software with the same working conditions as those in "Molecular Dynamics of Drug-Loaded Gel."

Table 1
Gelation Temperature of Blank Sol, LOF-Loaded Sol, and Ber-Loaded Sol

Sample	Blank Gel	LOF-Loaded Gel	Ber-Loaded Gel
Gelation temperature(°C)	34.8°C	35.0°C	34.5°C

Results and Discussion

Sol Dynamics

The formation mechanism of thermosensitive *in situ*—forming gel with F127 as gel matrix can be described as follows. F127 molecules exist in a monomer form at low temperatures. When the temperature rises, the hydrophobic PPO blocks form hydrophobic cores through van der Waals force and hydrophilic PEO blocks construct hydrophilic shell via hydrogen bonds; therefore, micelles are formed after the water molecules are taken off from the PPO chain segment.[22,23] The water molecules on the PEO chain segment get away as the temperature rises further, thus forming semisolid gel as the polymer entangles and packs closely.[6,24]

Based on aforementioned theory, it can be concluded that the addition of drug into sol had significant impacts on sol dynamics as seen in Figure 2. Ber-loaded gel required more time to slide down the same distance than the blank gel, whereas LOF-loaded gel accomplished sliding with a faster rate than the blank gel. The reasonable explanation was that the hydrophilic PEO blocks tangled more densely along with the involvement of poorly water-soluble Ber; therefore, it turned harder for the water molecules on PEO blocks to take off and further led to a lower sol dynamic rate. On the contrary, the addition of water-soluble LOF reduced the mutual entanglement of PEO blocks, thus resulting in a faster sol dynamic rate. It gave hints that water affinity of the drug can be the determinant factor that influences the sol dynamic rate of the drug-loaded sol.

Observation From Sol to Gel

The morphology from sol to gel was observed at different time intervals to vividly present the changes during gelation process and the morphologic differences between the blank sample and drug-loaded sample. As can be seen in Figure 3a, the blank sol had uniform surface without substances. However, there were spherical particles in LOF-loaded sol and larger amount of smaller needle-like particles in Ber-loaded sol, demonstrating that LOF and Ber were uniformly distributed in sol with different particle morphology. After the removing of some water molecules, the entanglement of F127 framework became clearer for these 3 samples. According to the gel state, the order of entangled intensity was Ber-loaded gel > blank gel > LOF gel, demonstrating that the drug had obvious impacts on entangled intensity of F127 sol and gel. The poorly water-soluble Ber improved entangled intensity and water-soluble LOF decreased the entanglement, which became the evidence for the aforementioned discussion of the sol dynamic study. Thus, it can be confirmed that the water affinity of the drug can be the determinant factor for the sol dynamic rate of drug-loaded sol owing to its impacts on entangled intensity of F127.

Gelation Temperature

The gelation temperatures of blank gel and drug-loaded gel are listed in Table 1. Generally, the gelation temperature of these 3 samples were in the range of 34°C to 35°C, which had the potential ability to reach the requirement of thermosensitive *in situ*—forming

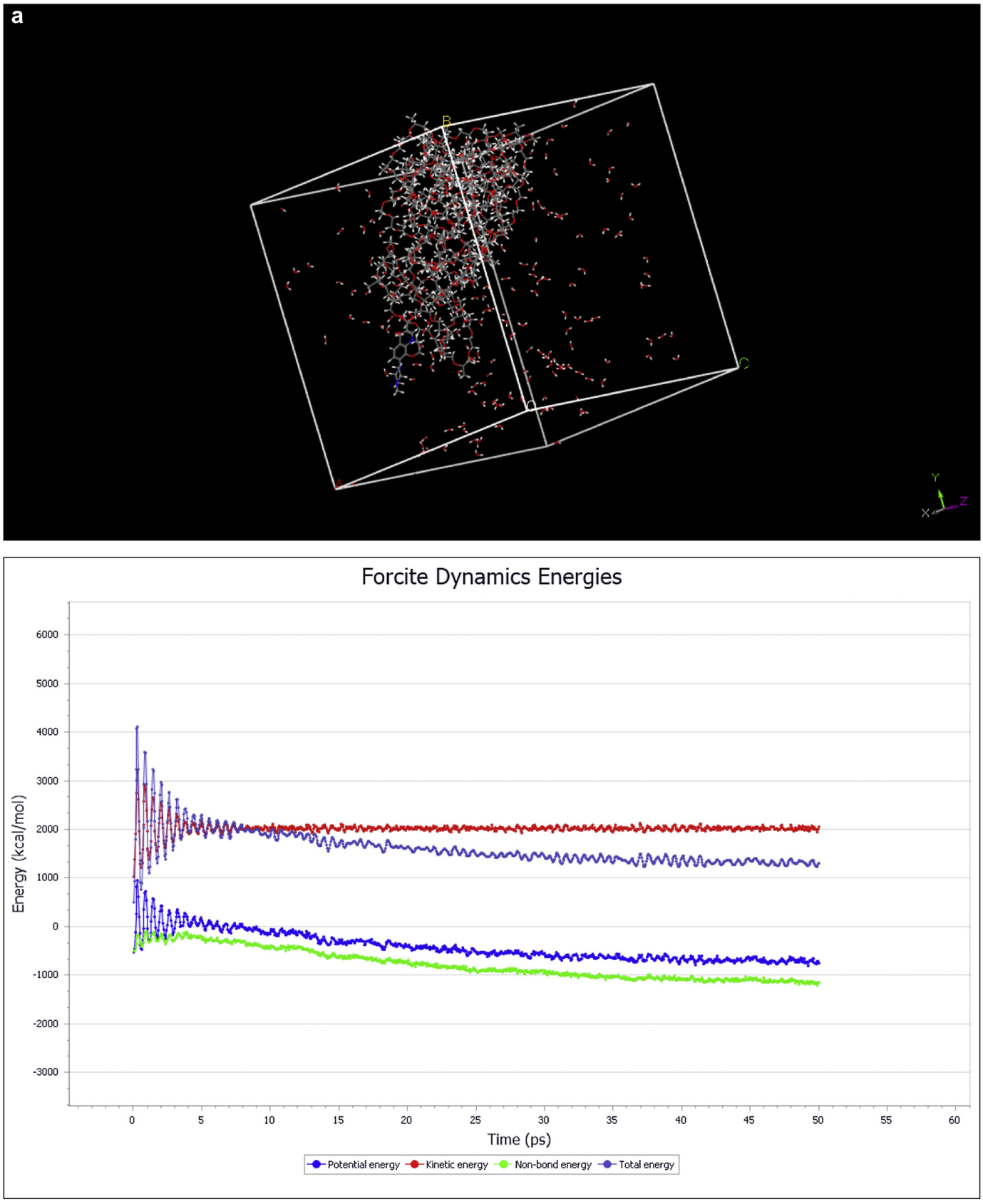
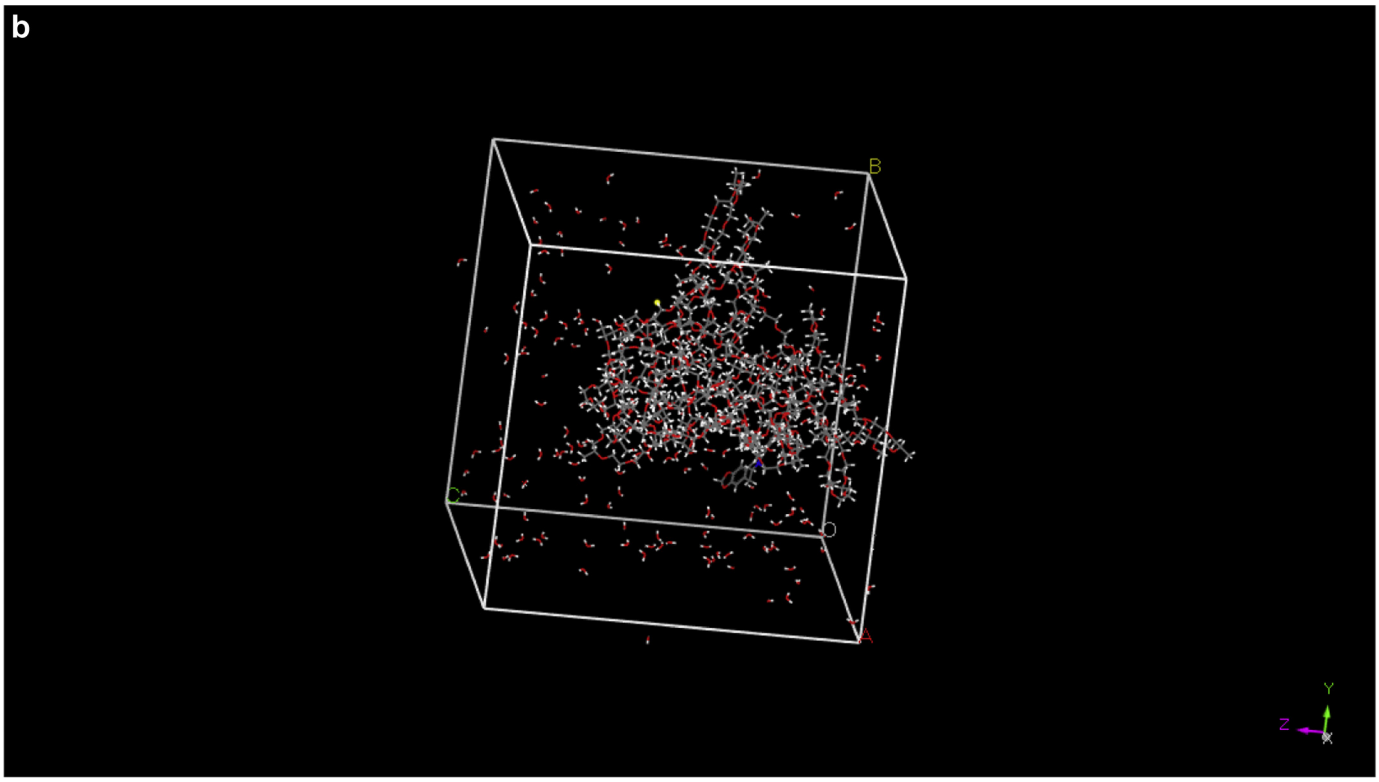
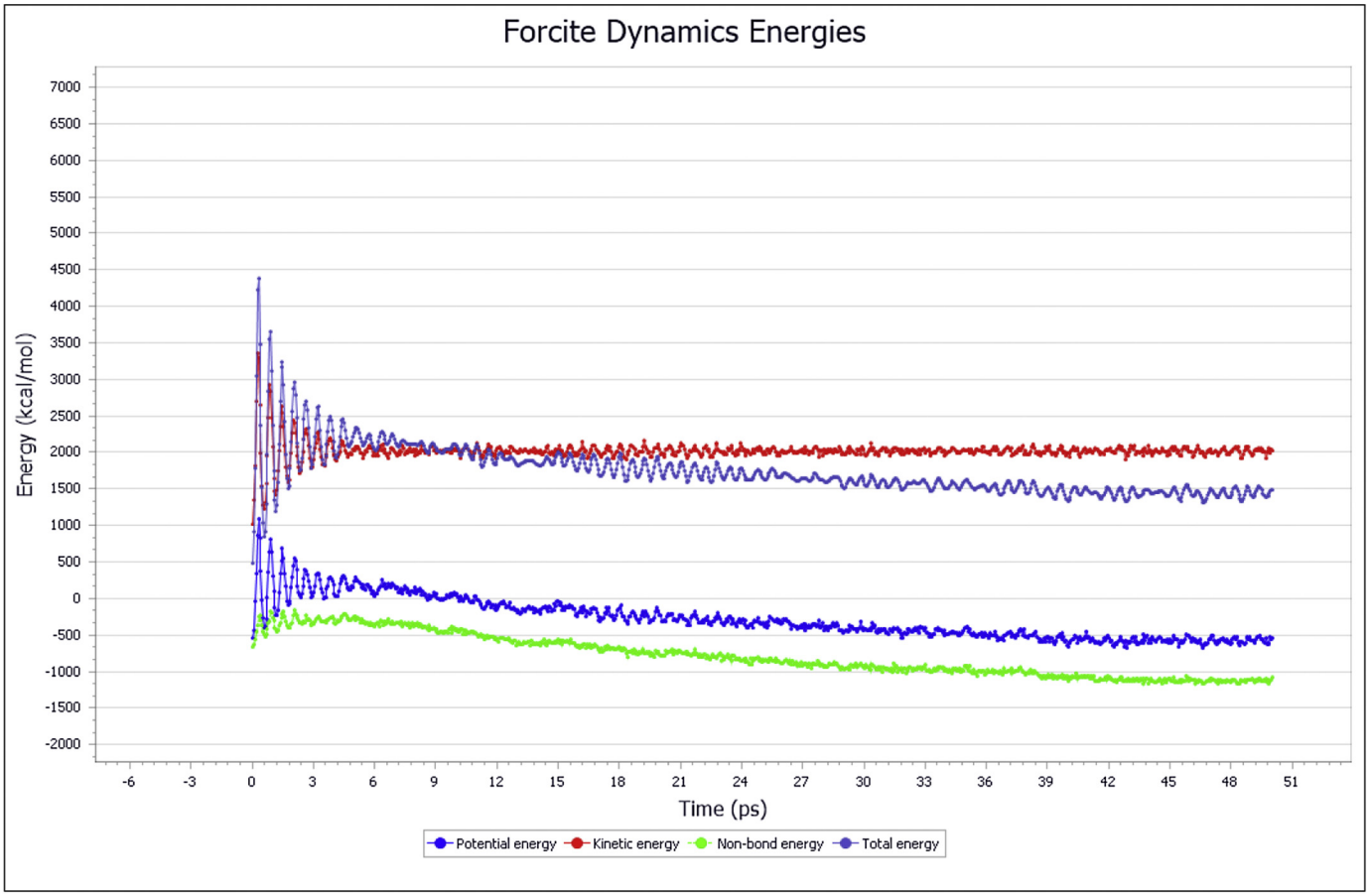


Figure 4. Molecular dynamic analysis of LOF-loaded sol and Ber-loaded sol.





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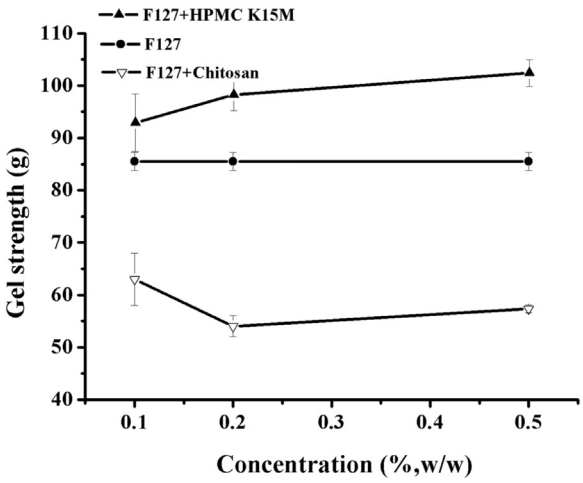

Figure 5. Gel strength of F127 sol, F127 + HPMC K15M sol, and F127 + chitosan sol.

ophthalmic gel (the gelation temperature should be about 35°C). After incorporating LOF into gel, the gelation temperature turned higher but not for Ber-loaded gel. These changes reflected that poorly water-soluble Ber improved entangled intensity of F127 and further reduced the sol dynamic rate, thus contributing to a lower gelation temperature. Similarly, water-soluble LOF decreased the entanglement of F127, leading to its faster sol dynamic rate and therefore higher gelation temperature. This correlation indicates the scientific interaction of these parameters, which provides great help in mastering the characteristics of drug-loaded gel.

Cornea Adhesion

The herein cornea adhesion analysis was conducted at room temperature, and therefore, the 3 samples existed at sol state, with the aim to magnify their differences in cornea adhesion and systemically study cornea adhesion from sol to gel. According to Figure 3b, LOF-loaded sol required less time than blank sol to slide down the same distance in the cornea, whereas Ber-loaded sol consumes the longest time among the 3, suggesting that the improved entangled intensity of F127 caused by poorly water-soluble Ber reduced its sol dynamic rate and further significantly enhanced cornea adhesion. As for LOF-loaded sol, the water-soluble LOF reduced entangled intensity of F127 and made its sol dynamic rate faster, resulting in weaker cornea adhesion than blank sol. Thus, it was clear that cornea adhesion of samples was generally in negative correlation with their sol dynamic rate.

As the temperature rose to almost 35°C, all these sol samples turned into gel and stayed onto the cornea. Washing the cornea using 35°C simulated tear liquid at the speed of 40μL/min until complete corrosion of gel sample reflected the residence time of gels on the eye. The results showed that the residence time of blank gel and LOF-loaded gel were 60 min, demonstrating that LOF had no impacts on residence effect of F127. Interestingly, the Ber-loaded gel with greatly enhanced cornea adhesion extended residence time to 100 min, which confirmed that the drug-excipient interactions can sometimes affect the function of the whole gel.

Gel Corrosion and Drug Delivery

Gel corrosion is defined as the changes or losses of characters/functions of gel in simulation. As a matter of fact, all gels have the nature of degradation and corrosion with various rates and different levels. Gel dissolution is hydrolyzed in aqueous medium and ruptured, thereby causing quality loss. This process belongs to membrane-less dissolution mechanism of the drug, and the weight loss reflects gel corrosion. Membrane-less dissolution mechanism can be described as the continuous corrosion of gel in the dissolution medium when gel directly contacts with the medium under vibration. Owing to the particularity of the ocular drug delivery system, the membrane-less dissolution model can simulate the eye environment with constant washing of tears and shearing action of blink. The result of Figure 3c demonstrates that the corrosion time of samples was in accordance with their residence time on the cornea. Furthermore, LOF-loaded gel and blank gel had constant corrosion behavior, whereas corrosion of Ber-loaded gel became slower, which was attributed to the impacts of drug on F127. Ber improved entangled intensity of F127 and reduced the sol dynamic rate, which further decreased its corrosion rate. With the corrosion of gel, drug was released gradually and similarly as the corrosion rate, and the dissolution rate of the Ber-loaded gel was lower than that of the LOF-loaded gel. To make further discussion about corrosion and drug release behaviors, cumulative corrosion amount and cumulative drug release were obtained by fitting analysis. The fitting curves of 2 drug-loaded gels were both logarithmic relation and had good relativity, illustrating that gel dissolution was the key factor that determined drug release. The impacts of the drug on this correlation were reflected from the slope and intercept. As Ber enhanced mutual entanglement between F127 molecules, gel corrosion and drug dissolution rates were reduced, leading to a logarithmic equation with a smaller slope and intercept.

Figure 4 revealed the dynamic energy of drug-loaded sol with the same amount of excipient and drug molecule. It was obvious that the final total energy of LOF-loaded sol (>−1000 kcal/mol) was higher than Ber-loaded sol (about −500 kcal/mol), confirming that the poorly water-soluble Ber improved entangled intensity and further reduced the sol dynamic rate owing to its lower total energy.

Table 2
Part of Molecular Information of HPMC K15M and Chitosan

Polymer	M (g/mol)	H Bond Donor	H Bond Receptor	Rotating Bonds
HPMC K15M	748.8	6	19	21
Chitosan	179.17	5	6	1

HPMC Enhanced the Strength of F127 Sol

According to Figure 5, the gel strength of F127 sol with the addition of HPMC K15M (0.1%, 0.2%, 0.5%, w/w) was obviously higher than F127 sol, and the gel strength of F127 sol with the addition of chitosan was quite lower than that of F127 sol, which was largely attributed to the mutual interaction forces formed between extra excipient and F127. HPMC K15M with a large number of hydrogen bonding acceptors and donors as well as rotating bonds formed (see Table 2) strong interaction forces with F127 when the molecular chain segments of HPMC K15M interspersed among the F127 network, resulting in higher hydrogen bonding density of sol and more condensed cross-linking of branched chains, thus contributing to stronger gel strength. On the contrary, chitosan (0.1%, 0.2%, 0.5%, w/w) with significantly fewer number of hydrogen-bonding acceptors and donors as well as rotating bonds reduced gel strength of F127 sol through loosening cross-linking of branched chains because of the lower hydrogen-bonding density of sol caused by weaker interaction forces formed between chitosan and F127.

Figure 6 showed the effect of existence of HPMC K15M and chitosan on F127 sol. The results demonstrated that the final total

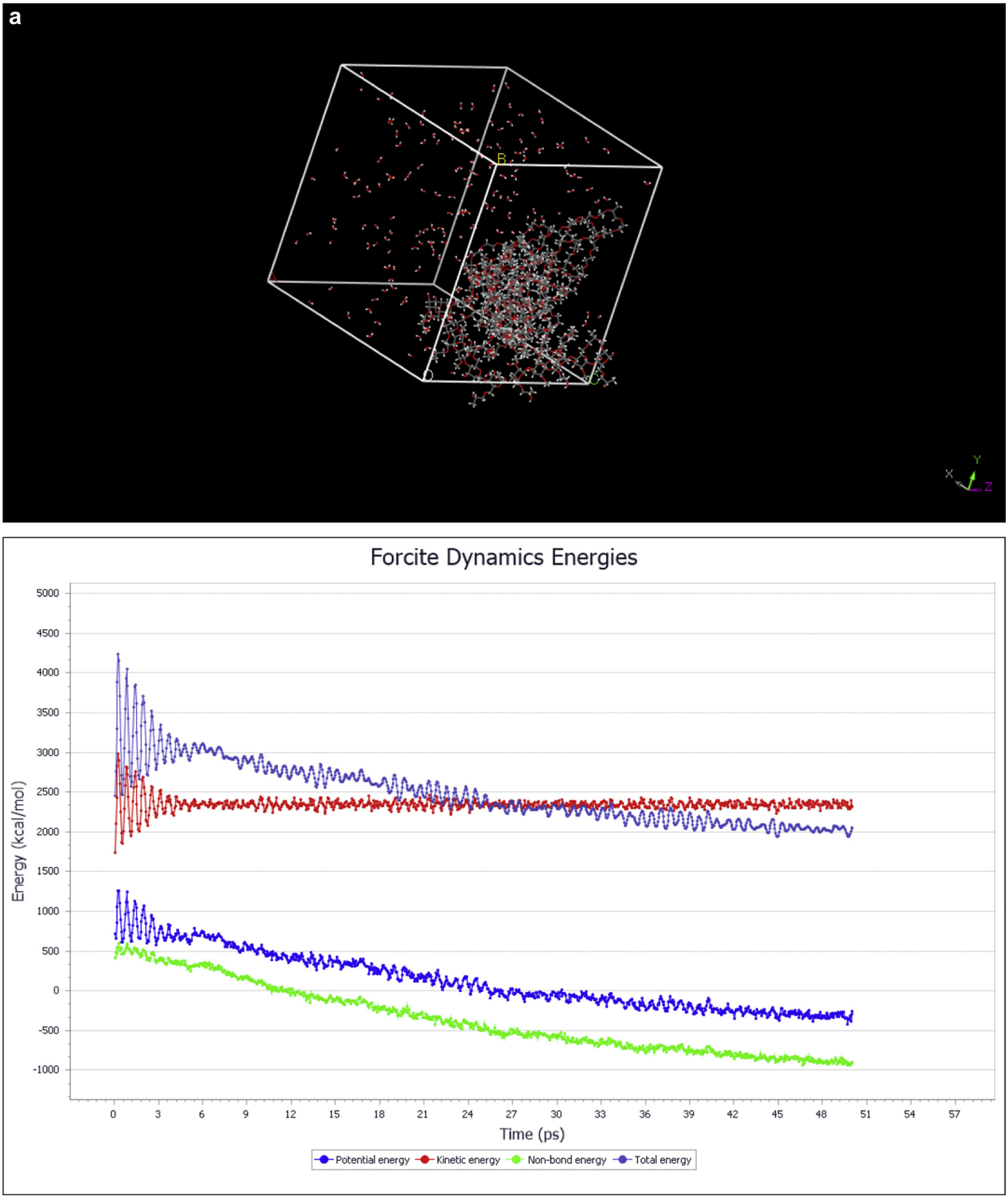

Figure 6. Molecular dynamic analysis of (a) F127 + HPMC K15M sol and (b) F127 + chitosan sol.

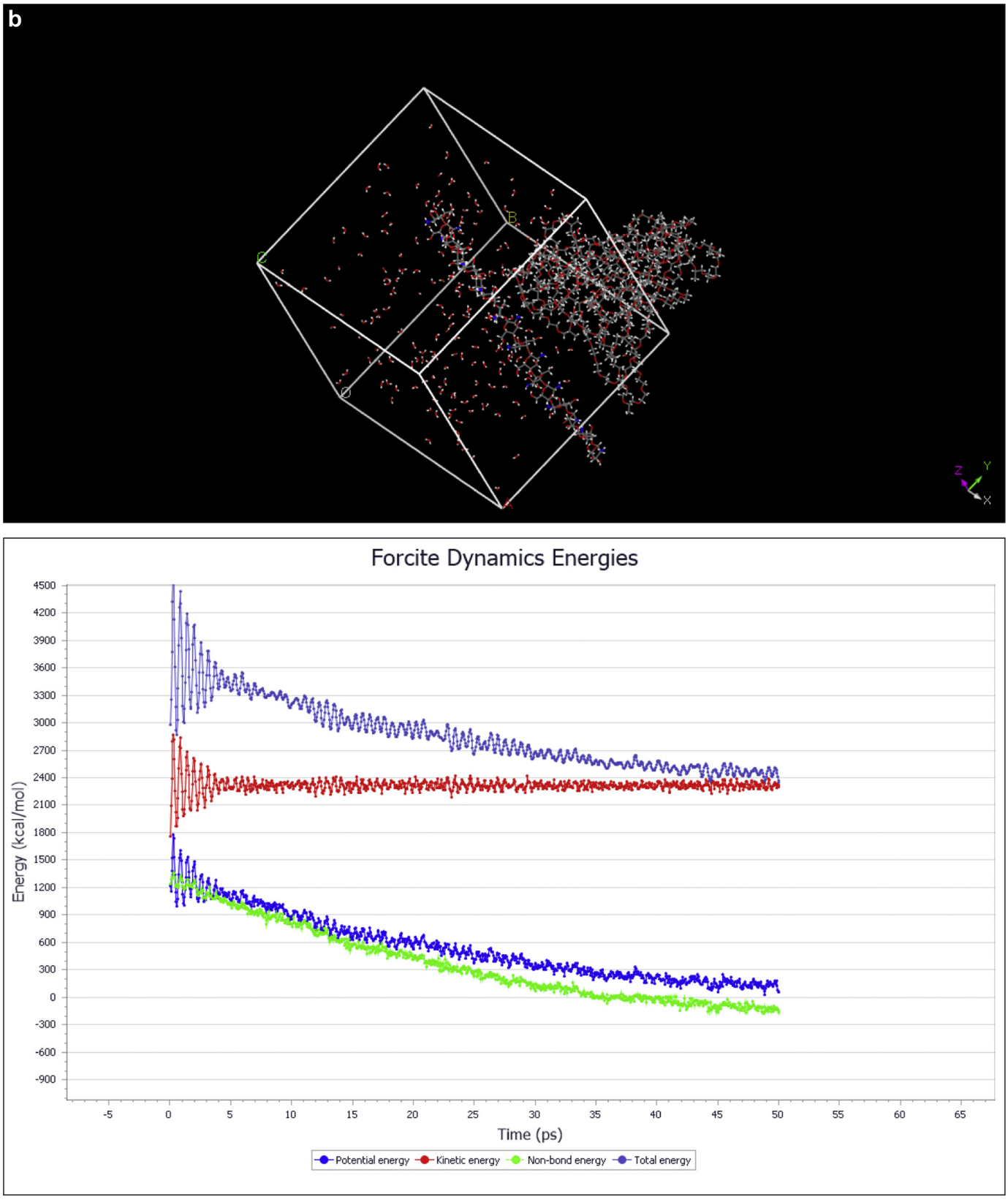

Figure 6. (*continued*).

energy of HPMC K15M in F127 sol (>−500 kcal/mol) was higher than that of chitosan in F127 sol (about 0 kcal/mol), confirming stronger interactions were formed between the F127 network and the molecular chain segments of HPMC K15M ascribed to its higher total energy and thus contributing to stronger gel strength with the addition of HPMC K15M.

Conclusion

The present work described facile and simple methods (load-bearing method, tilting plate method, and eye simulation method) to analyze and evaluate F127 in constructing thermosensitive *in situ*−forming ophthalmic gel. In conclusion, the drug affected the characteristics of F127 sol and F127 gel either with or without the cornea. The poorly water-soluble Ber improved entangled intensity of F127 and therefore reduced the sol dynamic rate, which contributed to lower gelation temperature, enhancement of cornea adhesion, and lower rate of gel corrosion together with drug release. Furthermore, gel dissolution was the key factor that determined drug release and the impacts of drug on this correlation can be learned from slope and intercept of fitting logarithmic equation. Importantly, the addition of HPMC K15M contributed to stronger gel strength because strong interaction forces were formed between HPMC K15M and F127 when the molecular chain segments of HPMC K15M interspersed among the F127 network, resulting in higher hydrogen bonding density of sol and more condensed cross-linking of branched chains. All the aforementioned results provide valuable instruction for designing and evaluating thermosensitive *in situ*−forming ophthalmic gel.

References

1. Rupenthal ID, Green CR, Alany RG. Comparison of ion-activated in situ gelling systems for ocular drug delivery. Part 1: physicochemical characterisation and in vitro release. *Int J Pharm*. 2011;411:69-77.
2. Sá Couto AR, Ryzhakov A, Loftsson T. Self-assembly of α-Cyclodextrin and β-Cyclodextrin: identification and development of analytical techniques. *J Pharm Sci*. 2018;107:2208-2215.
3. Cohen S, Lobel E, Trevgoda A, Peled Y. A novel in situ-forming ophthalmic drug delivery system from alginates undergoing gelation in the eye. *J Control Release*. 1997;44:201-208.
4. Cho K, Chung T, Kim B, et al. Release of ciprofloxacin from poloxamer-graft-hyaluronic acid hydrogels in vitro. *Int J Pharm*. 2003;260:83-91.
5. Li J, Zhao H, Okeke CI, et al. Comparison of systemic absorption between ofloxacin ophthalmic in situ gels and ofloxacin conventional ophthalmic solutions administration to rabbit eyes by HPLC-MS/MS. *Int J Pharm*. 2013;450:104-113.
6. Ma WD, Xu H, Wang C, Nie SF, Pan WS. Pluronic F127-g-poly(acrylic acid) copolymers as in situ gelling vehicle for ophthalmic drug delivery system. *Int J Pharm*. 2008;350:247-256.
7. Hatefi A, Amsden B. Biodegradable injectable in situ forming drug delivery systems. *J Control Release*. 2002;80:9-28.
8. Gurny R, Boye T, Ibrahim H. Ocular therapy with nanoparticulate systems for controlled drug delivery. *J Control Release*. 1985;2:353-361.
9. Srividya B, Cardoza RM, Amin P. Sustained ophthalmic delivery of ofloxacin from a pH triggered in situ gelling system. *J Control Release*. 2001;73:205-211.
10. Lindell K, Engström S. In vitro release of timolol maleate from an in situ gelling polymer system. *Int J Pharm*. 1993;95:219-228.
11. Desai SD, Blanchard J. Evaluation of pluronic F127-based sustained-release ocular delivery systems for pilocarpine using the albino rabbit eye model. *J Pharm Sci*. 1998;87:1190-1195.
12. Lavertu M, Filion D, Buschmann MD. Heat-induced transfer of protons from chitosan to glycerol phosphate produces chitosan precipitation and gelation. *Biomacromolecules*. 2008;9:640-650.
13. Alvarez-Lorenzo C, Bromberg L, Concheiro A. Light-sensitive intelligent drug delivery systems. *Photochem Photobiol*. 2009;85:848-860.
14. Mayol L, Quaglia F, Borzacchiello A, Ambrosio L, Rotonda MIL. A novel poloxamers/hyaluronic acid in situ forming hydrogel for drug delivery: rheological, mucoadhesive and in vitro release properties. *Eur J Pharm Biopharm*. 2008;70:199-206.
15. Liu Z, Li J, Nie S, Liu H, Ding P, Pan W. Study of an alginate/HPMC-based in situ gelling ophthalmic delivery system for gatifloxacin. *Int J Pharm*. 2006;315:12-17.
16. Asasutjarit R, Thanasanchokpibull S, Fuongfuchat A, Veeranondha S. Optimization and evaluation of thermoresponsive diclofenac sodium ophthalmic in situ gels. *Int J Pharm*. 2011;411:128-135.
17. Ur-Rehman T, Tavelin S, Gröbner G. Effect of DMSO on micellization, gelation and drug release profile of Poloxamer 407. *Int J Pharm*. 2010;394:92-98.
18. Li J, Li S, He Z. Molecular imprinting functionalized silica xerogel for selective recognition of levorotatory ofloxacin. *Mater Sci Eng C Mater Biol Appl*. 2017;71:999-1003.
19. Shi C, Tong Q, Fang J, Wang C, Wu J, Wang W. Preparation, characterization and in vivo studies of amorphous solid dispersion of berberine with hydrogenated phosphatidylcholine. *Eur J Pharm Sci*. 2015;74:11-17.
20. Pizones Ruiz-Henestrosa VM, Bellesi FA, Camino NA, Pilosof AMR. The impact of HPMC structure in the modulation of in vitro lipolysis: the role of bile salts. *Food Hydrocolloids*. 2017;62:251-261.
21. Ur-Rehman T, Tavelin S, Gröbner G. Chitosan in situ gelation for improved drug loading and retention in poloxamer 407 gels. *Int J Pharm*. 2011;409:19-29.
22. Yu S, Zhang X, Tan G, et al. A novel pH-induced thermosensitive hydrogel composed of carboxymethyl chitosan and poloxamer cross-linked by glutaraldehyde for ophthalmic drug delivery. *Carbohydr Polym*. 2017;155:208-217.
23. Banks SR, Pygall SR, Bajwa GS, Doughty SW, Timmins P, Melia CD. The influence of substituted phenols on the sol:gel transition of hydroxypropyl methylcellulose (HPMC) aqueous solutions. *Carbohydr Polym*. 2014;101:1198-1204.
24. Gratieri T, Gelfuso GM, Rocha EM, Sarmento VH, de Freitas O, Lopez RF. A poloxamer/chitosan in situ forming gel with prolonged retention time for ocular delivery. *Eur J Pharm Biopharm*. 2010;75:186-193.