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Study of the cohesion of TTCP/DCPA phosphate cement through evolution of cohesion time and remaining percentage

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Abstract Recent studies have revealed that the cohesion of calcium phosphate cement (CPC) might be a key property to ensure a safe and reliable clinical use of CPCs. Our study focused on the evolution of cohesion time and remaining percentage of TTCP/DCPA phosphate cement with changing factors in order to uncover the dependence of the cohesion of CPC on setting solutions and incubation liquids. Several factors were considered, including incubation liquids, concentration of setting solutions, powder to liquid (P/L) ratio, and polymer additive in setting solution. Results showed that the total ion osmotic pressure of both setting solutions and incubation solutions had significant influence on cohesion. Using physiological saline and simulation body fluid as incubation liquids indicated that the species of ions sometimes had much more profound effect on cohesion. Results of varying P/L ratios showed a great dependence on setting liquids, and a spot of water-soluble polymer additive was proved to be positive for cohesion.

Introduction

As one promising and practical biomaterial for hard tissue repairing, calcium phosphate cement (CPC) was well known for its high biocompatibility, easy-to-shape characteristic, and the capacity to self-setting under ambient conditions [1]. One unique property the CPC possessed is its ability to transform into a low-crystallinity hydroxyapatite and become hard at ambient temperature through a dissolution–precipitation mechanism [2–6] when mixed with physiological saline (PS) or other setting solutions. During implantation, CPC pastes are meant to harden through crystal entanglement even though they are in contact with streaming blood or body fluids. But before being delivered in the defected spot, the mixture should be in slurry state for injection convenience as well as being able to keep its integrity when in contact with body fluids or blood. The ability of a hydraulic paste to harden in an aqueous environment without releasing loose particles has been termed "cohesion" [7]. It could also be interpreted experimentally as the ability for cement to stay in one piece during setting process [8]. The disintegration of CPC when in contact with blood fluids could be an obstacle for its wide applications. For example, in the treatment of maxillo-facial defects and orthodontics, CPCs are likely to make contact with blood. If CPCs do not have strong cohesion, they may be easily disintegrated, hence defying the success of operations. Recent studies [9, 10] also showed that CPCs used for vertebroplasty might result in blood clotting, which was probably provoked by interfacial reactions between blood and solid particles released from CPCs when making contact with blood. Therefore, the study of disintegration is crucial in controlling and improving the performance of CPCs and broadening its application as well.

The time needed for CPC slurry to maintain integrity to resist the dispersion effects of streaming blood or other body fluids after being mixed is defined as cohesion time (CT), which is an important indicator employed to evaluate the cohesion. The time elapsed when the CPC putty became hard and possessed a certain surface strength from the time when it was prepared is usually defined as setting time. The remaining percentage (RP), the percentage of the remaining weight of CPC after partially disintegrated when

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immersed in incubation solutions in its original weight, is another important indicator of cohesion. It directly reflected the ability of CPCs to resist disintegration. Both CT and RP have significant meaning in characterizing the cohesion of CPCs. Short CT and high RP indicates strong cohesion. Fernandez et al. [11] proposed three methods to determine cohesion: (1) visual inspection; (2) measurement of geometrical size of the cement; (3) a setting time test (Gilmore needle) to assess whether resoftening of the cement occurred. The suggested methods were either imprecise or too difficult in operation. In this article, we proposed a new simple method different from the previous ones [7, 12]. By using this method, we can readily measure the CT and RP of CPC, which would provide us a much clearer understanding about the cohesion.

At present, many researchers are focusing on various additives in setting solutions, which can change either the cohesion or the rheological and mechanical properties of CPCs, such as sodium alginate [13], hydroxypropyl methylcellulose [14], sodium dextran sulfate [15] etc. It is known that CPCs are likely to disintegrate into small particles on early contact with aqueous solution, so the cohesion of CPCs is greatly affected by the interaction between cement paste and incubation liquids, which strongly depends on the compositions of CPCs and incubation liquids. Therefore, this study tries to provide a systematic investigation of the influence of compositions of cement paste and incubation liquids on the cohesion of TTCP/DCPA CPC by carefully tracing the two given indexes of CT and RP. We aim to give a better understanding on the mechanism and effects of the factors involved in the cohesion of CPCs and provide a reference to their clinical applications.

Materials and methods

Materials

CPC powder

The powder of CPC was made by mixing equimolar amounts of tetracalcium phosphate (TTCP: $Ca_4(PO_4)_2O$) and dicalcium phosphate anhydrous (DCPA: $CaHPO_4$). TTCP was prepared by calcination of an equimolar mixture of calcium carbonate ($CaCO_3$) (AR, Silian Chemical Co., Shanghai, China) and DCPA at 1,500 °C for 15 h followed by quenching. The sintered cake was first crushed by mortar and pestle until it passed through 40-mesh sieve. The powder was then milled in agate jars in ethanol ($\geq$99.6%) to obtain a median particle size of 4.75 μm with a specific surface area 0.6 m^2/g. DCPA was prepared by heating dicalcium phosphate dihydrate (DCPD: $CaHPO_4 \cdot 2H_2O$) (AR, Sinopharm Chemical Reagent Co., Ltd, China) at 120 °C for 12 h. Then it was ball milled in ethanol ($\geq$99.6%) for 30 h to obtain a median particle size of 0.346 μm with a specific surface area 6 m^2/g.

Setting solutions and incubation liquids

Distilled water (purified by Aquapro ultrapure water machine), neutral sodium phosphate solutions (NSPS) (Sinopharm Chemical Reagent, China), and dextran (Sinopharm Chemical Reagent, China) with different concentrations were used as setting solutions to study the effects of different setting solutions on the cohesion of CPC. Distilled water and NSPS of varying concentrations were also chosen as incubation liquids. Besides, Physiological Saline (PS) (0.9% NaCl solution) and Simulation Body Fluid (SBF) [16] (prepared in lab, Tris-hydroxymethyl aminomethane ($(HOCH_2)_3CNH_2$), 99.9+%, ultrapure grade, Sigma-Aldrich, Germany; other chemicals used were purchased from Sinopharm Chemical Reagent Co., Ltd, China) were also used as incubation liquids to investigate the effects of incubation liquids on cohesion.

Methods

The CPC paste was prepared by mixing the CPC powder and the selected setting liquids at a predetermined P/L ratio on a glass slab for approximate 30 s and the cement paste with a typical weight of 1 g was packed into a cylindrical mould. The mould was made by cutting off the front portion of a plastic syringe (12 mm in diameter) with the piston of the syringe still inside. After the paste was introduced into the mould from the open side, we pushed the piston with the open side leaning against a steel plate until the paste inside became 4-mm long. Then the syringe was lowered into incubation liquid vertically while the open side was immersed in incubation liquid. The setup for cohesion measurement applied in this study is illustrated in Fig. 1. We observed the disintegration by visual inspection immediately after immersion and used stopwatch to count CT. When there were no visible particles released from the CPC paste, we assumed this time as the end of disintegration, and the enduring time was considered as CT. After disintegration, the remaining paste still holding together within the syringe was collected and freeze-dried and the amount of dry powder was weighed and the RP was calculated according to the following equation:

$$\mathrm{RP}(\%) = \frac{\text{Weight of remaining CPC}}{\text{Weight of original CPC}} \times 100$$

For each combination of setting solutions and incubation liquids, we measured no less than five samples and all the results used are the mean values of those samples and deviation is indicated in error bars.

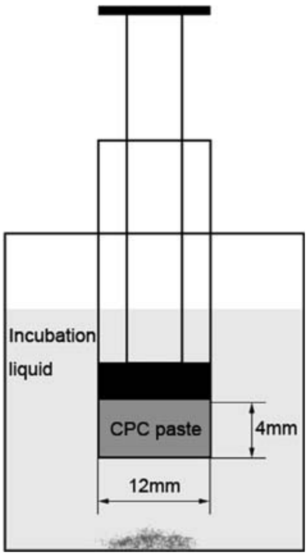

Fig. 1 Setup for cohesion measurement

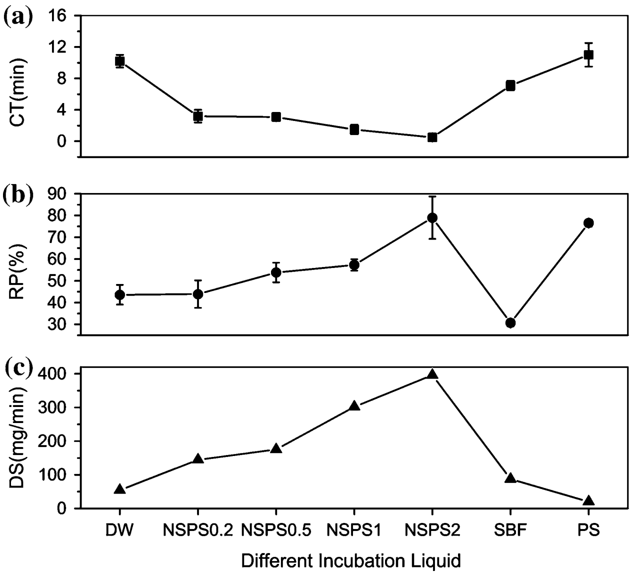

Fig. 2 Dependence of CT and RP on the incubation liquids as well as their concentrations. **a** Curve of CT. **b** Curve of RP. **c** Curve of DS. Distilled water, neutral sodium phosphate solution, simulation body fluid, and physiological saline were represented by DW, NSPS (concentration), SBF, and PS respectively

Results and discussion

Effect of incubation liquid

To investigate the effect of concentration of incubation liquids, distilled water was chosen as setting solution, P/L ratio was fixed at 4 g/L, and neutral sodium phosphate solution was picked as incubation liquid. With a varying concentration in the range from 0 (distilled water) to 2 mol/L, we investigated the evolution of CT and RP, and the results are shown in Fig. 2. NSPS with different concentrations were denoted as NSPS plus the concentration. It showed that with the increasing concentration of NSPS, CT (Fig. 2a) decreased from 10 min to no more than 1 min, falling dramatically at initial stage then becoming moderate when the concentration was above 0.2 M. RP (Fig. 2b) increased from 43.57% to 79.0%, whose trend was just converse, first climbing smoothly then elevating to maximum. Both indicated that the capacity of the cohesion of CPC became much stronger and more sufficient when concentration of NSPS increased. We also studied another two incubation liquids, SBF and PS, whose CT and RP were also charted in the same diagram with NSPS for comparison. Although both of them showed relatively higher CT, the RP was quite different. The SBF had a very low RP whereas PS showed a very high RP, which seemed inconsistent to each other. In addition, the disintegration speed (DS) of CPC paste during cohesion process was calculated according to the following formula:

$$\mathrm{DS} = \frac{(\text{Weight of original CPC} - \text{Weight of remaining CPC})}{\text{Cohesion time}}$$

The DS of CPC paste for different incubation liquids were also plotted in Fig. 2c. This curve showed a similar trend with the RP curve except that of PS, which had a very low disintegration speed.

Theoretically, the cohesion of CPC paste not only depends on the interaction energies within the cement paste (including (i) Van der Waals (attractive); (ii) electrostatic (repulsive); (iii) steric (repulsive)), but also largely on the osmotic pressure between setting solution and incubation liquid [7]. And the osmotic pressure depends on the difference between the concentration of interstitial liquid and the incubation liquid. If the total solute concentration in incubation solution is lower than that in the interstitial liquid, then the flow of molecules from incubation solution into the cement will be larger than in the opposite direction. This flow results in the swelling of the cement surface and hence the decohesion of the cement. If the solute concentration in the solution is larger than in the interstitial liquid, the cement surface tends to be depleted from its liquid, hence increasing the cement cohesion. Therefore, the cohesion of a cement paste is expected to be better in neutral sodium phosphate solution than in pure water. In our study, the setting solution, or the interstitial liquid was distilled water. Therefore, with increasing concentration of NSPS, its osmotic pressure became increasingly larger than that of interstitial liquid, and then the flow of water molecules from the cement into the NSPS was larger than that in the opposite direction which resulted in better cohesion.

Distilled water, SBF, and PS are commonly used incubation liquids. In this study, SBF has an ion concentration of 0.308 M with a tris(hydroxymethyl)-aminomethane concentration of 0.05 M, and the ion concentration of PS is 0.304 M. Both have an ion concentration higher than distilled water but lower than any NSPS used in this experiment, so in term of osmotic pressure, both of them should have a CT and RP value between that of distilled water and NSPS0.2 (>0.4 M). The CT of SBF given in Fig. 2 was in this range and it was lower than that of PS due to its higher ion and molecular concentrations. The CT of PS was a little higher than distilled water, which was acceptable if experimental error was considered (error bar). A great difference was manifested in their RP. SBF had the smallest RP of approximately 30% while PS had a profound high RP of nearly 77%, which may result from the different ion species in these two incubation liquids. Besides comparative content of sodium and chloride ion, one major difference between SBF and PS was that SBF contained more other ions like K^+, Mg^{2+}, Ca^{2+}, HCO_3^-, HPO_4^{2-} etc, and 0.05 M tris-hydroxymethyl aminomethane. Tris-hydroxymethyl aminomethane was a commonly used buffer solution, and played a role in stabilizing the pH of the SBF solution, which may result in the difference of pH values of these two incubation liquids when CPC was immersed, and have remarkable effect on the electrostatic forces existed in the CPC paste and hence changed the cohesion.

Effect of the concentration of setting liquid

Sodium phosphate solution is always used as an ingredient acting as an accelerator of setting reaction. In our experiments, SBF was incubation liquid, P/L ratio was predetermined at 4 g/L, and NSPS with varying concentrations was chosen as setting solution to investigate the effect of the concentration of setting liquid on cohesion. The results were plotted in Fig. 3. When the concentration of NSPS increased from 0 to 1.0 M, CT first increased to 15 min at 0.2 M, and was followed by dramatic decrease to 2 min at 1.0 M. Correspondingly, RP first decreased to almost zero at 0.2 M and then jumped to a high level.

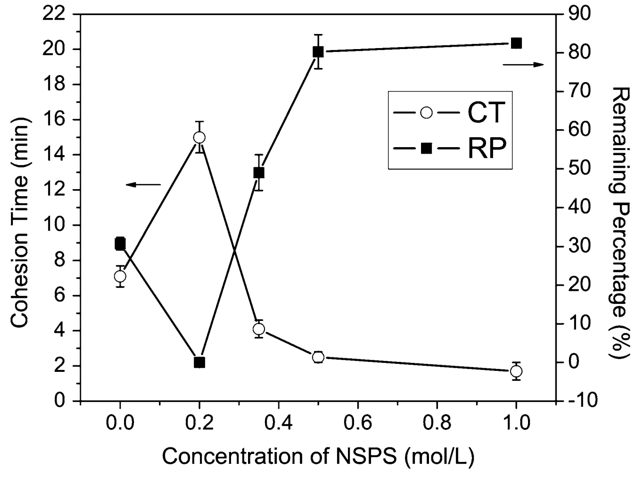

Fig. 3 Dependence of CT and RP of CPC on the concentration of NSPS when SBF was applied as incubation liquid. Curve of CT (○) and curve of RP (■)

According to Bohner [7], an increase of setting solution concentration has both positive and negative effects on cohesion. It would increase the ionic strength of interstitial liquid, which was expected to enhance cohesion by reducing thickness of the electrical double layer, and hence decrease the electrostatic interactions. However, it would also increase the osmotic pressure and hence reduce cohesion. So this study provided a good support to this mechanism. The cohesion process was dominated by the osmotic pressure when the concentration of NSPS was below 0.2 M. When it was higher than 0.2 M, the cohesion was dominated by the electrostatic interactions. The combination of these two effects resulted in the maximum CT and the minimum RP at 0.2 M, which was corresponding to the worst cohesion. From CT and RP, the cohesion of this putty was much better at higher NSPS concentrations.

Ishikawa [17] conducted a detailed study on the effects of neutral sodium hydrogen phosphate on setting reaction and mechanical strength of hydroxyapatite putty, which showed that a shorter setting time was observed for the HAP putty containing higher concentrations of NSPS. It also showed that when the concentration of NSPS was higher than 0.2 M, the mechanical strength of HAP putty would increase with increasing concentration of NSPS. We might conclude that increasing NSPS concentration could be beneficial to both the cohesion and mechanical strength of this HAP putty. However, with the acceleration of setting reaction, the setting time decreased, which might increase the difficulties of operation.

Effect of powder to liquid ratio

Frequently, the P/L ratio of CPC was chosen between 3 and 5 g/mL in literature. In this study, two groups of experiments have been conducted using 0.2 M NSPS and distilled water as the setting solution, respectively, and SBF was used as incubation liquid. Results (Table 1) showed that varying P/L ratios of CPC exhibited different trends in two setting solutions. When 0.2 M of NSPS was used as setting solution, both CT and RP of the CPC indicated a decrease in cohesion with increasing P/L ratio. In contrast, when distilled water was used as setting liquid, the CT and RP showed an increase of cohesion with increasing P/L ratio.

Powder to liquid ratio can directly influence the distance between particles in CPC paste. According to the Lennard–Jones equation [11],

$$\Phi = \xi x^{-12} - \beta x^{-6},$$

where Φ is the force and x is the distance between particles. ξ and β are two constants. Increasing P/L ratio can shorten

Table 1 The effect of P/L ratio (incubation liquid: SBF)

Setting solution	P/L (g/mL)	CT (min)	RP (%)
0.2 M NSPS	3.3	1.6 ± 0.1	66.4 ± 1.5
	4	Almost disintegrated within 2 min	
	5		
Distilled water	3.3	Almost disintegrated within 2 min	
	4	7.1 ± 0.6	30.7 ± 1.6
	5	13.9 ± 1.9	72.5 ± 8.2

the distance of particles and hence strengthen the force and interactions between particles, and enhance the cohesion consequently. When distilled water was the setting solution, RP increased with increasing P/L ratio, which confirmed that increasing P/L ratio did enhance the integration of CPC particles although the CT also slightly increased. However, a reversed trend was observed when 0.2 M of NSPS was used as the setting solution. Compared with the results in Fig. 3, the cohesion of CPC at this concentration was the worst due to the combination effect of osmotic pressure and electrostatic interactions. Increasing P/L ratio from 4 to 5 g/mL was useless to make any significant improvement on the cohesion. Only when the P/L ratio was lowered to 3.3 g/mL, it could gain a better cohesion. It was assumed that the electrostatic interactions between particles were weakened with increasing setting solution when 0.2 M of NSPS was used as setting solution, which caused the desorption of the absorbed ions in CPC [7].

Liu et al. [18] has determined the dependence of setting time on the P/L ratio, using a rheological method. They measured the oscillatory time sweep curves of CPC paste at different P/L ratios and obtained the evolution data of elastic modulus (G′) with time. Theoretically, the elastic modulus will show a turning point at the setting process, from which we can determine the setting time. The results showed that the setting time decreased when P/L increased. This calculation agreed well with the setting time obtained by the experiment of Vicat method, which also showed that the setting time was shortened as the value of P/L rose.

Effect of dextran: its concentration and molecular weight

Combining polymer additives into CPC pastes proved to be an effective solution to adjust the rheological properties (e.g., viscosity, fluidity, injectability, and stability) [19–21] as well as improving the mechanical strength of CPC [22]. Polymers to be combined should be water soluble, nontoxic, and easy to degrade. In this study, two groups of experiments were designed to investigate the effect of polymer content and molecular weight on the cohesion of CPC paste. SBF was used as incubation liquid, P/L ratio was fixed at 4 g/mL, and dextrans with different molecular weights were used as polymer additives. A weight content range of dextran (molecular weight: 500,000) varied from 0 to 10%, and three typical molecular weights (fixed weight content 0.6%) have been investigated and the results of the CT and RP were included in Fig. 4. Figure 4a showed that with increasing weight content of dextran in setting solution, the CT first declined slightly followed by a rapid increase to more than 10 min, and then maintained at a high level without significant change when the weight content rose from 0.6% to 10%. However, the increase of molecular weight had a subtle positive effect on the cohesion of CPC. Figure 4b showed the dependence of RP

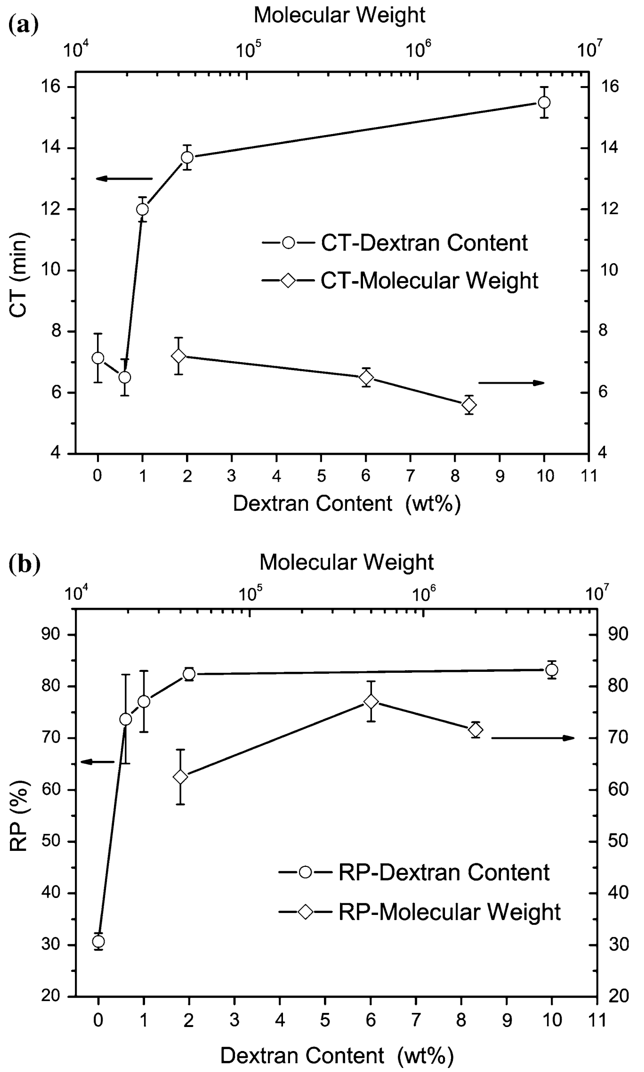

Fig. 4 The effect of dextran content and its molecular weight on the CT and RP. **a** Dependence of CT on the dextran content (○) and molecular weight (◇). **b** Dependence of RP on the dextran content (○) and molecular weight (◇). The molecular weight used in studying dextran content was 500,000 and the weight percentage of dextran used in studying molecular weight was 0.6%

on dextran content and its molecular weight. When dextran content was above 0.6 wt%, RP kept above 70%, and changes in molecular weight also showed insignificant influence on RP. The result of RP with increasing dextran content was very similar with the report of Ishikawa et al. [23]. They also measured the change of setting time with polymer content. The results showed that the setting time increased from 5.3 min to 31.8 min when the concentration of polymer additive increased from 0 to 10 wt%. Bohner [24] studied the dependence of setting time on the concentration of citrate, and a linear increase of setting time was found when the concentration increased.

We noticed during experiment that the volume of CPC slurry with dextran was much larger than that of CPC slurry without dextran. It indicated that dextran absorbed a lot of water molecules through hydrogen bonding and attached to the surface of CPC particles to enlarge the steric effect, which eventually resulted in the swelling of CPC slurry. The distance between particles extended, and hence the Van der Waals force decreased and so did the cohesion. This explanation held true when the dextran content was above 0.6 wt%. But more attention should be attached to the comparison between 0 wt% and 0.6 wt% dextran. At 0.6 wt%, the CT was the lowest and the RP was nearly 75% which was much higher than that at 0 wt%. It indicated that a relative low content of dextran was helpful to improve cohesion.

We measured the change of the viscosity of CPC paste with the concentrations of dextran solution, using Rheometer Mars II (Haake, Germany), with shearing rate varying from 0.01 s^{-1} to 100 s^{-1}. The test was conducted immediately after the preparation of CPC slurry and each test took about 3 min. Figure 5 gave the dependence of viscosity on different dextran content, which showed that the viscosity had the largest value when the dextran content was 0.6 wt%. Anything other than 0.6 wt% dextran content would decrease the viscosity. A possible explanation is that an appropriate content of water-soluble polymer like dextran can deprive the CPC paste of water so as to decrease the effective liquid mixed with CPC, which is equivalent to increasing the P/L. Polymer dosage which is higher or lower than this critical value would be adverse to the cohesion because of the limited water in CPC paste and the increase of interstitial osmotic pressure (caused by rising content of polymer). However, with the increasing content of dextran, RP increased too. It did not mean that the cohesion of CPC paste enhanced. This is because the particles were stabilized in the one, two, or three-dimensional networks of dextran, and the more dextran there was, the more effective this network was in stabilizing these particles. The polymers were more likely to be suspended in aqueous without falling down, and the particles which were supposed to disintegrate into incubation liquids were bound by dextran chains and could not disintegrate in a remarkable speed.

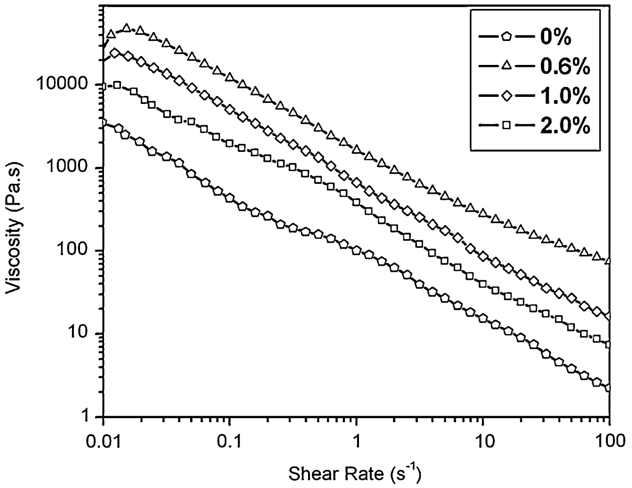

Fig. 5 The viscosity curves of CPC paste with different dextran weight content (0%, 0.6%, 1.0%, 2.0%). Shear rate varies from 0.01 s^{-1} to 100 s^{-1}

Though the dependence of CT and RP on the molecular weight was not significant, it did have some implications. Both the CT and the RP of CPC were not sensitive to the change in molecular weight. Increasing molecular weight could slightly decrease the CT, and so did the cohesion. However, extremely high molecular weight was not favored due to the decrease in RP, which might be caused by the sedimentation of dextran.

Conclusion

Various factors, including incubation liquids, concentration of the setting solutions, P/L ratio and polymer additives with different weight contents and molecular weights have been investigated. The dependences of CT and RP of CPC paste on those variations were studied and uncovered by a simple but reliable method which could obtain data of CT and the RP simultaneously. We found that the total ion osmotic pressure of both setting solution and incubation solution had a profound impact on the cohesion of CPC paste. Low interstitial and relatively high incubation liquid osmotic pressures were favored in strengthening cohesion. Besides, the species of ions sometimes had much more significant effects on the electrostatic interactions of CPC, which would cause large fluctuations in CT and RP. P/L ratio did have an influence on the force between particles, but results in this study showed that it was not the determinative factor. Addition of water-soluble polymer in the setting solution was complicated because both positive and negative factors are involved in this system, but it was obvious that there existed an optimum loading of polymer in setting solution. This study provided a good reference to controlling the CT and RP of CPC pastes for their clinical applications.

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