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Note

A novel, simple method to simulate gelling process of injectable biodegradable *in situ* forming drug delivery system based on determination of electrical conductivity

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ABSTRACT

The purpose of the present study was to develop a novel, simple and determination-of-electrical-conductivity-based method to trace the gelling process of injectable biodegradable *in situ* forming organogels after administration. The electrical conductivity of pH 7.4 PBS solution with different amount of N-methyl-2-pyrrolodone (NMP) and drug-free organogel formulation contained 0.6 mL NMP were determined at 37 °C, respectively. The electrical conductivity of PBS solution was linearly proportional to the amount of NMP. Organogel contained 0.6 mL NMP in PBS solution showed a descending of electrical conductivity as time runs, while the value of electrical conductivity was almost a constant at 7.58 ms/cm after 110 min, which was nearly equaled to the electrical conductivity of 0.6 mL NMP in PBS solution (7.59 ms/cm). This data indicated that the diffusion of NMP caused the descending of system electrical conductivity and NMP completely diffused from organogel after 110 min, which led to the constant electrical conductivity. Meanwhile, photographs of organogel showed that the gel formed from periphery to center gradually, and totally formed after 110 min. The diffusion terminal point of NMP from organogel could be perfectly anticipated and controlled by this method. Consequently, this electrochemical method had visually simulated the gelling process and located gelling time of organogel in medium solution by measuring variation of electrical conductivity.

Injectable biodegradable *in situ* forming drug delivery system has been studied as injectable sustained-release drug delivery for many years. Among these, hydrogels has been widely used as parenteral drug depot systems (Gong et al., 2009a,b; Wei et al., 2009). However, in order to improve the biocompatibility of injective *in situ* gelling systems, many organogels have been used as attractive drug delivery in the last few decades due to their non-toxic degradation production, loading different properties drugs, and raising the stability of biomolecules drugs (Motulskya et al., 2005; Vinitiloiu et al., 2007). Organogel drug delivery systems were semisolid formulations composed of organogelator, hydrophilic solvent, vegetable oil and active ingredient, in which an organic liquid phase (vegetable oil) was immobilized by a three-dimensional network composed of self-assembled, intertwined gelator fibers (Vintiloiu and Leroux, 2008). Organogelators were mostly low molecular weight molecules with the capacity of self-assembling in organic liquids at low concentration. Above the gelling concentration, noncovalent intermolecular interactions led to various supramolecular entangled structures ranging from fibers to strands or tapes. The resulting three-dimensional network prevented the solvent from flowing and showed solid-like properties to the system (Abdallah and Weiss, 2000).

The presence of hydrophilic solvents such as N-methyl-2-pyrrolodone (NMP), alcohol, dimethyl sulfoxide (DMSO), 2-pyrrolidone, etc., partially disrupted the interactions between gelator molecules, which maintained the formulation in a sol state and allowed injection of the formulations at room temperature. Such a result attributed to the fact that the hydrophilic solvent reduced the extent of intermolecular interaction between the organogelator molecules by creating H-bonding with the latter, thereby inhibiting gelation upon cooling (Plourde et al., 2005). So it was called gelation inhibitor. Upon administration, the gelation inhibitor gradually diffused into the surrounding aqueous environment and organogelator molecules self-assembled to create tridimensional network, which caused the gelling of organogel. Until hydrophilic solvent completely diffused from the formulation, organogel formed *in situ* began to degrade *in vivo* and release their payload slowly. Therefore, it can be concluded that the time of hydrophilic solvent, when it entirely diffused in the surrounding tissues, is just the final time of organogel forming and the starting point

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of the degradation *in vivo*. The diffusing rate of gelation inhibitor directly results in the formation of injectable organogel. Thus, the hydrophilic solvent can be described as the "switch" to control the gelation process. Organogels formed *in situ* were traced by the dynamic diffusing course of hydrophilic solvent. In other words, the speed and degree of diffusion would radically determine the property of organogel system. Consequently, studying the diffusing process of the hydrophilic solvent can not only clarify the gelling procedures of organogels and explain the degradation of organogels (Wang et al., 2010), but also predict drug release *in vivo* (Plourde et al., 2005). In spite of this, by now there have been no explicit methods to trace the diffusing process of hydrophilic solvent. It is very important to develop a method to accurately monitor the diffuse behavior and locate the terminal point. Therefore, our present study is to develop a novel and convenient electrochemical method to simulate the gelling procedures and gelling time of organogels.

Electrical conductivity of medium solution [phosphate buffer solution (PBS), pH 7.4, 20 mL] was measured at 37 °C by DDS-11A conductivity meter (Lida, Shanghai, China). As FDA-approved hydrophilic solvent, NMP (Bodi, Tianjin, China) was selected to accomplish the research. Different volumes of NMP were dropped into medium solution and the electrical conductivities (EC) of system were measured, respectively. The change of conductivity with different amount of NMP was determined so as to find the corresponding point at which the NMP completely diffused from the following organogel formulation. The organogelator N-lauroyl-L-alanine methyl ester (LAM) used in this study was synthesized in our laboratory (Motulsky, 2005). The drug-free organogel system was prepared as reported previously (Jia et al., 2009), LAM (300 mg) was dissolved in 1 mL soybean oil (Beiya, Tieliing, China) at 70 °C, and NMP (0.6 mL) was added to it, finally the mixture was stirred and preheated at 37 °C. Control group was prepared as above without NMP. 20 mL PBS was added in baker in 37 °C water bath, and then the conductivity of medium solution was measured. Organogel and control group were transferred into bag filter, and put in medium solution, respectively. The conductivities of systems were determined every 5 min. The change of conductivity by time was determined.

Electrical conductivity (EC) is a measurement of a material's ability to conduct an electric current. EC estimates the total amount of dissolved ions in the solution. The more ions are in the solution, the more conductive it is, the higher an electrical current appears in the solution as a result. In our experiment, numbers of ions in solution are descent as the amount of NMP increases, which induces the loss of electrical conductivity. It may be due to the presence of nitrogen atom in NMP molecule that can attract ions in the solution, leading to the descending of EC.

It can be seen from Fig. 1 that the variation of electrical conductivity depended on the amount of NMP in medium solution. As NMP increases, electrical conductivity of system decreases. It is clear that electrical conductivity is linearly proportional to the amount of NMP. The linearity is demonstrated by plotting the electrical conductivity (y) against the amount of NMP (x). The calibration curves are obtained by linear regression analysis. Equation of linear regression is $\kappa = -1.064M + 8.231$ ($R^2 = 0.9949$). The plotted calibration curves and correlation coefficients >0.99 confirm that the calibration curves are linear over the amount range of 0–1.0 ml. This suggests that the electrical conductivity can reflect the amount of NMP added in the medium solution. Therefore, measurement of electrical conductivity can indicate the amount of NMP in the solution. For the organogel system in PBS solution, conductivity change is due to the NMP diffusion as time goes by. Thus, this method can be used to trace the diffusion course of NMP from organogel system in PBS solution and locate the end point of diffusion. Taking into account that the amount of NMP in the following organogel formulation is 0.6 mL, it is emphasized that the electrical conductivity value of 0.6 mL NMP in 20 mL PBS solution is 7.59 ms/cm.

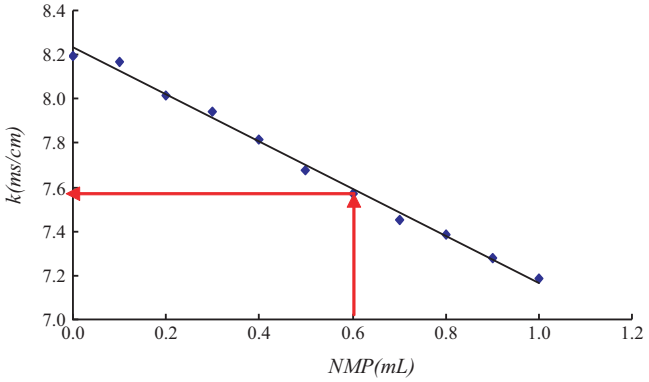

Fig. 1. Calibration curve of electrical conductivity with NMP in PBS.

The diffusion of NMP from organogel was investigated and the electrical conductivity of system was measured each 5 min. The results of organogel and control group were listed in Table 1. Electrical conductivity of medium against time was presented in Fig. 2. Fig. 3 showed the dynamic gelation course of organogel along the diffuse time.

It was clear that the electrical conductivity of control group was essentially constant at 8.25 ms/cm. This was consistent with the previous determination. But as to the organogel group (with 0.6 mL NMP), the electrical conductivity was decreasing as time goes by. It was distinct that the decrease of electrical conductivity was mainly caused by the diffusing of NMP, which can be explained from the photos (Fig. 3). Fig. 3A was the photography of organogel at the 0th minute. It was a clear injectable sol, where the conductivity was also 8.25 ms/cm. After it was put into the PBS solution, the diffusion of NMP was started and gel began to form. In the 30th minute (Fig. 3B), gel was formed from periphery to center, showing an opaque edge and transparent center. Taking the results of conductivity into account (7.95 ms/cm), the reason was that NMP was only partly diffused out at this time. With the diffusion of NMP, the

Table 1
Values OF electrical conductivity (EC) of control group and organogel at different time ($n = 3$).

Time (min)	EC of control (ms/cm)	EC of organogel (ms/cm)
0	8.25 ± 0.000	8.26 ± 0.044
5	8.25 ± 0.006	8.16 ± 0.044
10	8.26 ± 0.010	8.09 ± 0.040
15	8.25 ± 0.006	8.05 ± 0.049
20	8.25 ± 0.025	8.04 ± 0.032
25	8.24 ± 0.030	8.01 ± 0.035
30	8.25 ± 0.031	7.95 ± 0.040
35	8.27 ± 0.015	7.94 ± 0.046
40	8.26 ± 0.017	7.91 ± 0.035
45	8.27 ± 0.015	7.90 ± 0.021
50	8.25 ± 0.029	7.88 ± 0.031
55	8.25 ± 0.035	7.87 ± 0.029
60	8.26 ± 0.038	0.84 ± 0.046
65	8.26 ± 0.017	7.80 ± 0.035
70	8.26 ± 0.015	7.76 ± 0.06
75	8.27 ± 0.015	7.71 ± 0.021
80	8.26 ± 0.015	7.71 ± 0.031
85	8.27 ± 0.012	7.69 ± 0.036
90	8.26 ± 0.006	7.65 ± 0.038
95	8.26 ± 0.006	7.63 ± 0.032
100	8.27 ± 0.020	7.61 ± 0.032
105	8.27 ± 0.021	7.59 ± 0.031
110	8.26 ± 0.015	7.58 ± 0.020
115	8.27 ± 0.010	7.58 ± 0.015
120	8.27 ± 0.010	7.58 ± 0.015
125	8.27 ± 0.010	7.57 ± 0.012
130	8.27 ± 0.006	7.58 ± 0.015

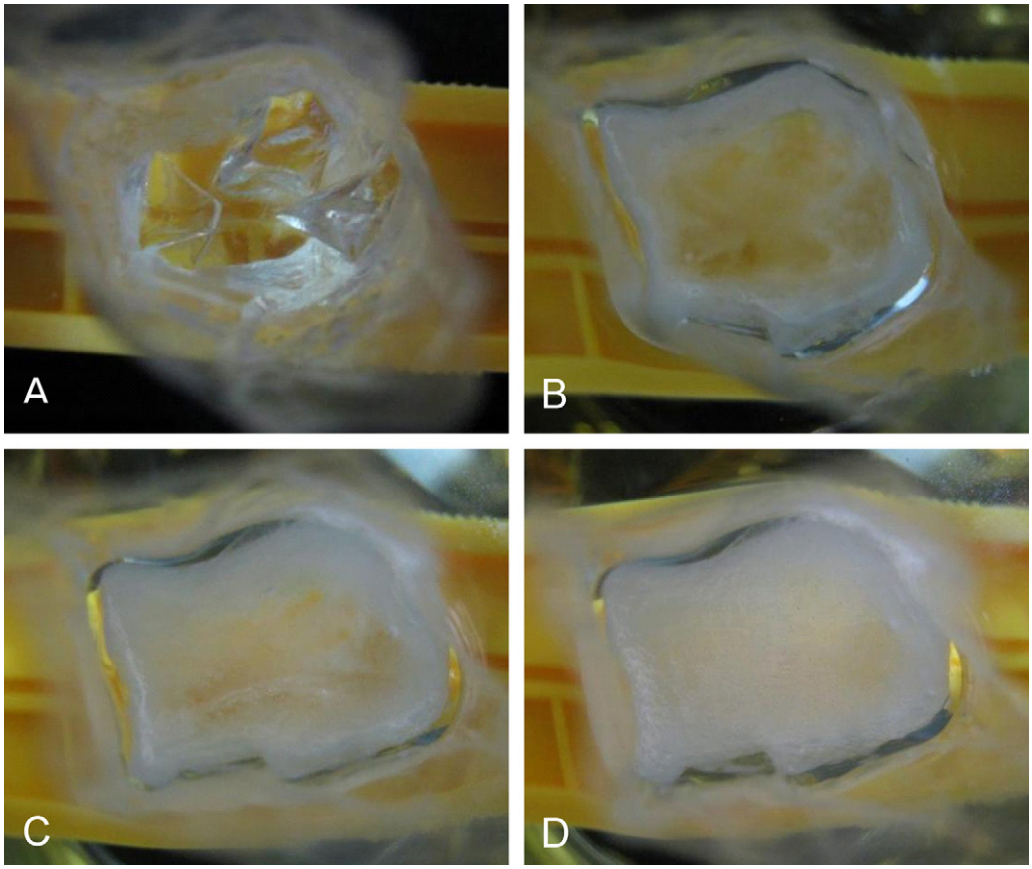

Fig. 3. Photographs of organogel in PBS solution at 37 °C at the 0th minute (A); 30th minute (B); 70th minute (C); 110th minute (D).

area of opaque gel was gradually increased, and the conductivity fell to 7.76 ms/cm. It was very clearly that there was only a little part of organogel was kept transparent according to Fig. 3C. Until the 110th minute, gel became fully opaque, which indicated that NMP was completely diffused out and organogel was completely formed (Fig. 3D). According to the conductivity, after 110 min, the value of electrical conductivity maintained almost steady at 7.58 ms/cm. As to the equation of linear regression, this value was exactly equaled to the electrical conductivity of 0.6 mL NMP in 20 mL PBS solution (7.59 ms/cm).

In conclusion, as NMP diffused into the surrounding aqueous environment, organogelator molecules began to form intermolecular hydrogen bond, which caused the transition from sol to gel. Until 110 min, NMP added in organogel absolutely diffused into medium, while organogel was completely formed *in situ* and would begin to degrade *in vivo* and release their payload slowly. So, it can be concluded that the final gelling time of LAM organogel in PBS at 37 °C is 110 min. In other words, this time is just the starting point of the degradation *in vivo*. The method based on electrical conductivity can accurately monitor the diffuse behavior and locate the terminal point so as to clarify the gelling procedures of organogels, to explain the degradation of organogels, and to predict drug release *in vivo*.

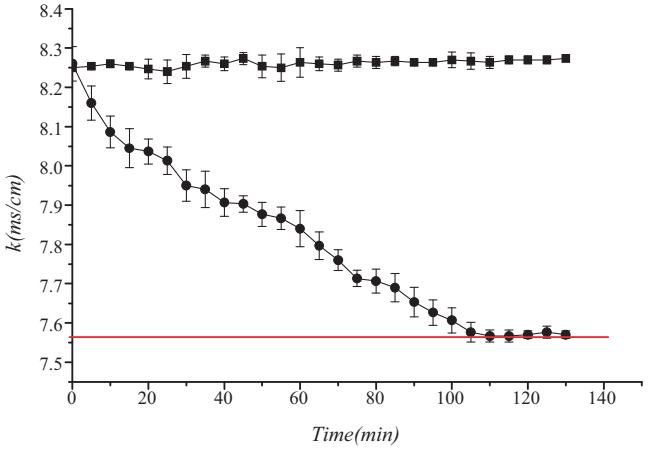

Fig. 2. Electrical conductivity curves of control and organogel in PBS with time. Organogel without NMP (■) and organogel contained NMP (●).

In this study, a novel, simple and accurate method to simulate the gelling process of organogel is developed. The addition of gelation inhibitor NMP causes the descending of electrical conductivity in the system due to its attraction of ions. Meanwhile, electrical conductivity is linearly proportional to the amount of NMP. The diffusion end of NMP from organogel is perfectly anticipated and controlled. It provides fundamental basis for further studies on organogel forming *in situ*, degradation *in vivo* and drug-release behavior. Therefore, this electrical conductivity method can be used to trace the gelling, degrading and drug-releasing course of organogel drug delivery systems.

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