**Jing GUAN[1*], Peng CHENG[1,2], Shujie HUANG[1], Zhizhong LI[1], Jimin WU[1],
Ping GAO[3], Xiaohui WANG[3], Hua ZHANG[2]**

Academy of Military Medical Sciences (1), Tianjin Polytechnic University (2) , Tianjin Institute of Medical and Pharmaceutical Sciences (3)

Mathematic Simulating of Levofloxacin Release from Chitosan Nano/Microparticles

Abstract. Chitosan based levofloxacin nano/microparticles were prepared by ionotropic gelatin and emulsion crosslinking technique. Their in vitro release profiles showed that microparticles had better sustaining delivery property than nanoparticles. The best fitting kinetics for nanoparticles and microparticles were First Order and Diffusion-Relaxation model respectively. The result suggests that levofloxacin delivery from nanoparticles was mainly controlled by diffusion process only, and that from microparticles was dominated by both diffusion and relaxation process.

Streszczenie. Mikro- i nanoelementy lekarstwa levofloaxin były wytwarzane techniką sieciowania emulsji. Dla lepszego dopasowania kinematyki opracowano model matematyczny. Stwierdzono że nanocząstki dostarczają materiał przez dyfuzję podczas gdy mikrocząstki wykorzystują też relaksację. (Matematyczna symulacja lekarstwa levofloaxin w postaci nano- i mikroelementów)

Keywords: Chitosan, Levofloxacin, Nano/Microparticles, Mathematic Simulating.
Słowa kluczowe: nanoelementy, mikroelementy, model matematyczny

Introduction

Chitosan (CS) is a biodegradable natural polymer with great potential for pharmaceutical applications due to its biocompatibility, high charge density, non-toxicity and mucoadhesion [1]. CS nano/microparticle delivery systems allow drug release to be carefully tailored to the specific treatment site through the choice and formulation of various drug–polymer combinations. The total dose of medication and the kinetics of release are the variables, which can be manipulated to achieve the desired goal. Using different technologies and by varying the experimental parameters, CS nano/microparticles can be developed into specific therapy systems which will provide the desired release profiles. CS nano/microparticle delivery systems may increase the life span of active constituents and control the release of bioactive agents, including antibiotics, anticancer agents, proteins, peptide drugs and vaccines [2].

As an antibiotics with broad-spectrum antibacterial activity, levofloxacin (LOF) is effective to either gram positive or gram negative bacteria, and is widely used for controlling urinary tract, respiratory tract, skin and soft tissue infections. However, it requires frequent dosing to maintain therapeutic effect due to its short biological half-life and greatly varying pharmaceutical concentrations of blood. Sustained delivery LOF preparation can help to maintain effective drug concentration, reduce dosing times, improve compliance, and thus optimize drug therapy [3].

Many researches on CS based LOF microsphere delivery systems have been carried out during the past few years, most of which focused on the preparation parameters and therapy levels, including spherical geometry, release profile, and tissue targeting [4-6]. Among these studies, numerical simulating of LOF delivery have been undertaken because it can provide further insight into drug release mechanisms and contribute to elucidating the influences of various physical parameters [7].

Compared with CS microparticle delivery system, nanoparticles have smaller particle size, larger surface to volume ratios, and therefore stronger tissue targeting and better controlled release properties [8]. However, few studies on CS based LOF nanoparticles have been carried out so far.

In this paper, CS nanoparticles and microparticles loaded with LOF were prepared by ionotropic gelatin and emulsion crosslinking technique based on our previous study [9], and their physical properties, such as particle size, span and loading capacity (LC) were tested. Then release properties of particles were examined and cumulative release curves vs. time were protracted. Subsequently those release data were simulated by different mathematic models in order to obtain the best fit kinetic equation. Finally the corresponding release mechanism from nano/microparticles was discussed based on their release behavior and the best fit kinetic model.

Materials and methods

MATERIALS. LOF (Zhejiang East Pharmaceutical Co., China); CS (molecular weight: 98000; deacetylation degree> 87%, Shandong Aokang Biological Technology Co., China); Tripolyphosphate (TPP)（Sigma, USA）; Liquid paraffin, Petroleum ether, Ethanol (Tianjin Yingdaxigui Chemical Reagent Factory, China); Span-80 (Shanghai Volkswagen Pharmaceutical, China); Glutaraldehyde (50%, Tianjin Bodi Chemical Co., China); Glacial acetic acid, Hydrochloric acid (Tianjin Chemical Reagent Co., China)

PREPRATION OF NANOPARTICLES BY IONOTROPIC GELATIN. $80mg$ of LOF was put into $20ml$ of $2mg/ml$ CS acetic solution under $500rpm$ until dissolution. This solution was then dropped into $5ml$ of $2mg/ml$ TPP solution, and pH was adjusted into the range of 4.5 to 6. The nanoparticles were obtained by lyophilization. The ionotropic gelatin process can be described as follows:

CS   TPP
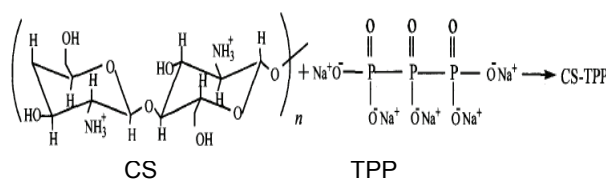
PREPARATION OF MICROPARTICLES BY EMULSION CROSSLINKING. $50ml$ of liquid paraffin and $2ml$ of Span-80 were mixed by stirring at room temperature to form oil phase. $150mg$ LOF was added into $10ml$ of 1% acetic acid solution containing $300mg$ CS with agitation to form water phase. Then total of $10ml$ water phase was dripped into above oil phase at a speed of 40-60 drops per minute. The mixture was emulsified at $1500rpm$ stirring speed until stable emulsion formed. Then certain amount of glutaraldehyde was dropped into the system and stirred continuously at room temperature for $1h$. After that, the suspension was washed by petroleum ether and then by

ethanol, and finally lyophilized. The crosslinking reaction can be described as follows:
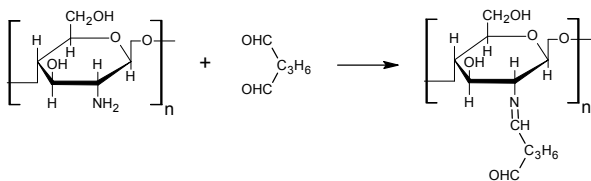
STANDARD EQUATION OF LOF. $5mg$ LOF was weighed accurately and placed into $50ml$ volumetric flask. Then pH 7.4 PBS solution was added into the flask to make $0.1mg/ml$ LOF stock solution. Then the stock solution was diluted into series of standard solutions with LOF concentrations of $3\mu g/ml$, $6\mu g/ml$, $9\mu g/ml$, $12\mu g/ml$ and $15\mu g/ml$ respectively. The absorbance of each solution at $293nm$ was determined by UV spectrophotometer with pure PBS as blank. Then LOF standard equation was regressed.

SIZE AND *SPAN* OF NANO/MICROPARTICLES. Nano/Microparticles were diluted by ethanol to form dispersion. The *Size* of those particles was measured by laser particle size analyzer at 25 ℃. The S*pan* was calculated by the following formula:

$$(1) \qquad Span = \frac{D_{90} - D_{10}}{D_{50}}$$

where D_{90}, D_{50}, and D_{10}- the particle size for which 90%, 50% and 10% of the particles are smaller than this volume respectively.

LC OF NANOPARTICLES. The *LC* of nanoparticles were determined by separation of nanoparticles from the medium containing free drug by centrifugation at $14000rpm$ for $30min$. The absorbance of free LOF in the supernatant was measured by UV at $293nm$ and the amount of LOF was calculated according to standard equation. The sedimentation was then lyophilized and weighed. The *LC* of the nanoparticles can be calculated as following formula:

$$(2) \qquad LC = \frac{T - F}{M} \times 100\%$$

where: *T*-total mass of LOF added into CS solution, *F*-free LOF mass in the supernatant, *M*-the total mass of nanoparticles.

LC OF MICROPARTICLES. Microparticles were grinded, weighed, and then dissolved in $10ml$ of $0.1M$ hydrochloric acid solution for 7 days with intermittent agitation. Then the solution was centrifuged at $5000rpm$ for $10min$, after that, $1ml$ of supernatant was drawn into $250ml$ volumetric flask and diluted. The absorbance at $293nm$ was measured as above. The total LOF mass in the particles can be determined according to LOF standard equation and its *LC* value can be calculated according to the following formula:

$$(3) \qquad LC = \frac{M1}{M2} \times 100\%$$

Where: M_1 -the total LOF mass in the microspheres, M_2-the total mass of microspheres.

IN VITRO RELEASE BEHAVIOR. $0.01g$ nanoparticles were placed in a dialysis bag and put into a cell containing $100ml$ of pH 7.4 PBS solution under the condition of 37 and $100rpm$ agitation. Subsequently series of $1ml$ dialysis solutions were withdrawn into $100ml$ volumetric flask at specific time points ($0.5h$, $1h$, $3h$, $5h$, $7h$, $10h$, $12h$, $24h$, $48h$, $72h$, $120h$) and diluted. Then their absorbencies at $293nm$ were determined as above. The release dosage at each moment was calculated and drawn into cumulative release curve. Similarly, microparticles were performed the same

procedure except the sampling points occurring at $0.5h$, $1h$, $3h$, $5h$, $7h$, $12h$, $24h$, $48h$, $72h$, $120h$, $168h$ and $240h$ respectively. The release dosage at each moment was calculated and drawn into cumulative release curve as well.

MODELING DRUG RELEASE. The LOF release data of CS nanoparticles and microparticles were simulated to different kinetic models shown in Table 1. The release rate constants were calculated by Origin8.0 software.

Table 1. The Kinetic Models of Drug Release

Models	Q(t)
Zero Order	$Q(t)=Q_0+kt$
First Order	$Q(t)=Q_0(1-e^{-kt})$
Higuchi	$Q(t)=Q_0+kt^{1/2}$
Ritger-Peppas	$Q(t)=Q_0+kt^m$
Diffusion-Relaxation	$Q(t)=Q_0+k_1t^{1/2}+k_2t$
Hixson-Crowell	$Q(t)=Q_0+k_1t+k_2t^2+k_3t^3$
Diffusion-Erosion	$Q(t)=Q_0+k_1t^{1/2}+k_2t+k_3t^2+k_4t^3$

Results and Discussion

SIZE, *SPAN* AND *LC* OF PARTICLES. The standard equation of LOF can be regressed as following:

$$(4) \qquad A = 0.0697C - 0.0563 \quad (r^2=0.9907)$$

Where: *A*-the absorbance of solution at $293nm$; *C*-the concentration of LOF, r^2 -the related factor. The 0.9907 of r^2 value implies that the regressed equation matched well with experimental data, and above equation can be used as standard formula to assess the drugcontents.

Based on above standard equation, *LC* values of nanoparticles and microparticles were evaluated as 6.1% and 21.7% respectively. Their particle sizes were 140 *nm* and 1.59 *μm* with S*pans* of 0.95 and 0.99 for nanoparticles and microparticles respectively (Table 2).

Table 2. *Size*, *Span* and *LC* of Nano/Microparticles

Preparation Method	Size (*μm*)	*Span*	*LC* (%)
Ionotropic Gelatin	0.14	0.95	6.1
Emulsion Crosslinking	1.59	0.99	21.7

IN VITRO RELEASE BEHAVIOR OF NANOPARTICLES. The relationship between percentage of cumulative release and releasing time of nanoparticles was demonstrated in Figure 1, where the scattered dots represent experimental data. It can be observed that the initial release was fast with cumulative release percentage up to 10% at $0.5h$, 21.7% at $3h$, 50% at $7.5h$, 60% at $10h$, 72% at $12h$, while the subsequent release became slower gradually with about 85% at $24h$, 90% at $56.5h$. The release profile indicated that the nanoparticles could release LOF gradually up to 3 days.

Several kinetic models relating to drug release from matrices, selected from the most important mathematical models, including Zero Order, First Order, Higuchi, Ritger-Peppas, Diffusion-Relaxation, Hixson-Crowell, and Diffusion-Erosion, were employed to simulate the release data and labeled as real lines in Figure 1. The real line of Figure 1 (b) matched much better with experimental data than that of others, which indicates that the First Order was the best fit model for nanoparticles.
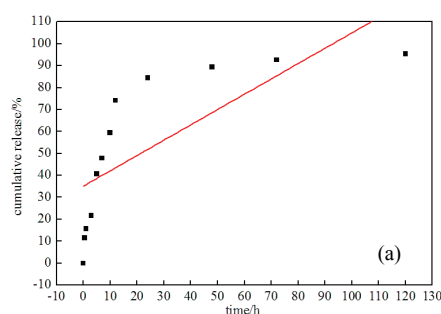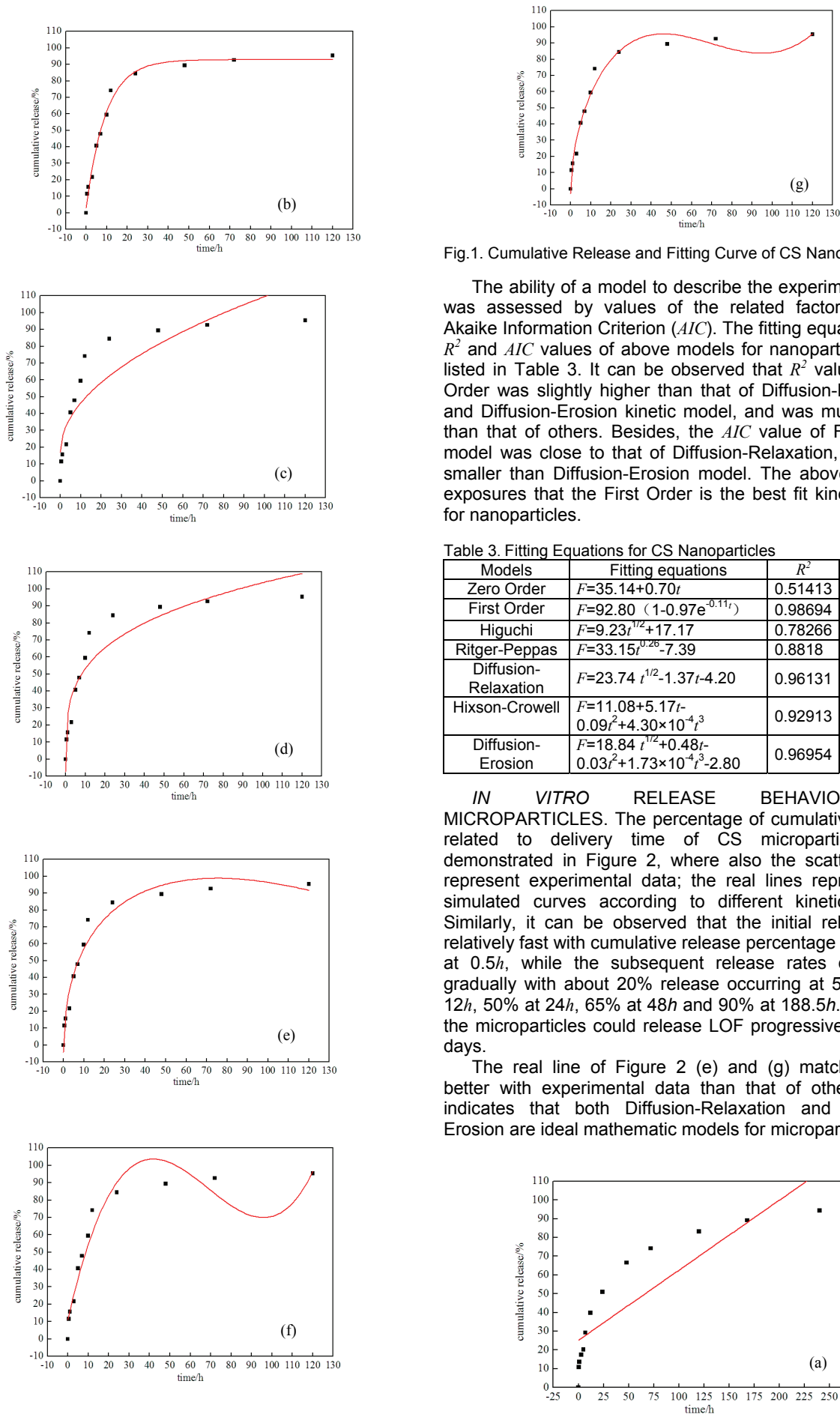
Fig.1. Cumulative Release and Fitting Curve of CS Nanoparticles

The ability of a model to describe the experimental data was assessed by values of the related factor (R^2) and Akaike Information Criterion (AIC). The fitting equations with R^2 and AIC values of above models for nanoparticles were listed in Table 3. It can be observed that R^2 value of First Order was slightly higher than that of Diffusion-Relaxation and Diffusion-Erosion kinetic model, and was much higher than that of others. Besides, the AIC value of First Order model was close to that of Diffusion-Relaxation, but much smaller than Diffusion-Erosion model. The above analysis exposures that the First Order is the best fit kinetic model for nanoparticles.

Table 3. Fitting Equations for CS Nanoparticles

Models	Fitting equations	R^2	AIC
Zero Order	$F=35.14+0.70t$	0.51413	0.4380
First Order	$F=92.80（1-0.97e^{-0.11t}）$	0.98694	9.3258
Higuchi	$F=9.23t^{1/2}+17.17$	0.78266	4.7085
Ritger-Peppas	$F=33.15t^{0.26}-7.39$	0.8818	7.8168
Diffusion-Relaxation	$F=23.74\,t^{1/2}-1.37t-4.20$	0.96131	8.9413
Hixson-Crowell	$F=11.08+5.17t-0.09t^2+4.30\times10^{-4}t^3$	0.92913	8.6161
Diffusion-Erosion	$F=18.84\,t^{1/2}+0.48t-0.03t^2+1.73\times10^{-4}t^3-2.80$	0.96954	17.1450

IN VITRO RELEASE BEHAVIOR OF MICROPARTICLES. The percentage of cumulative release related to delivery time of CS microparticles was demonstrated in Figure 2, where also the scattered dots represent experimental data; the real lines represent the simulated curves according to different kinetic models. Similarly, it can be observed that the initial release was relatively fast with cumulative release percentage up to 10% at 0.5*h*, while the subsequent release rates decreased gradually with about 20% release occurring at 5*h*, 40% at 12*h*, 50% at 24*h*, 65% at 48*h* and 90% at 188.5*h*. However, the microparticles could release LOF progressively up to 7 days.

The real line of Figure 2 (e) and (g) matched much better with experimental data than that of others, which indicates that both Diffusion-Relaxation and Diffusion-Erosion are ideal mathematic models for microparticles.
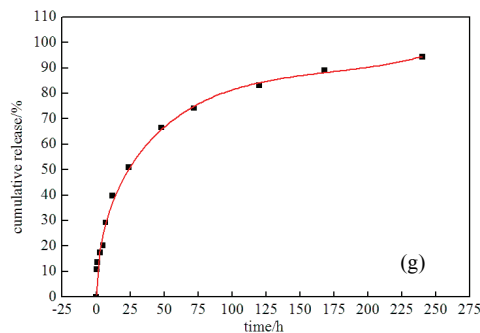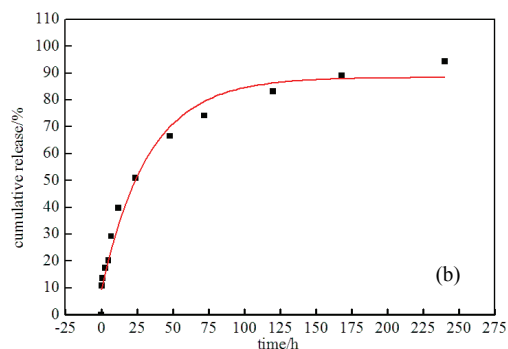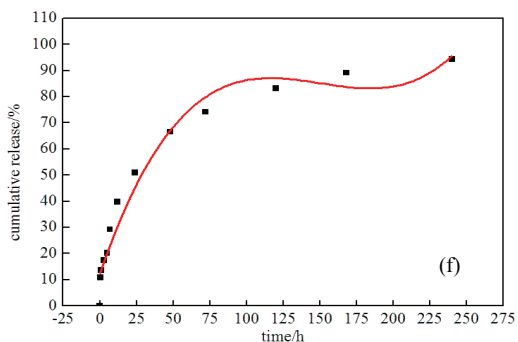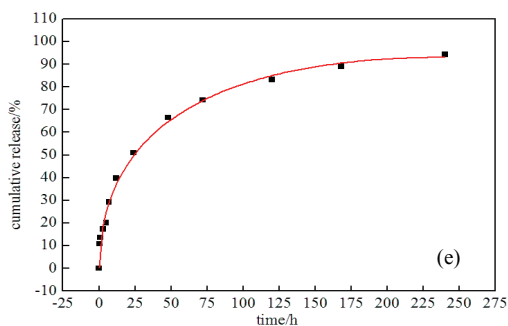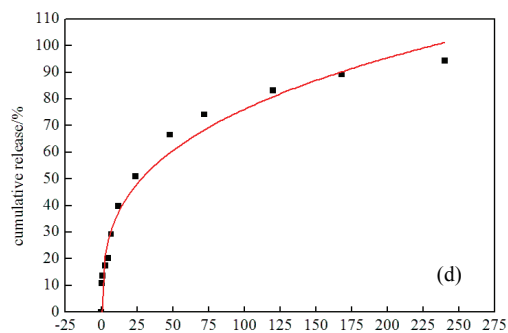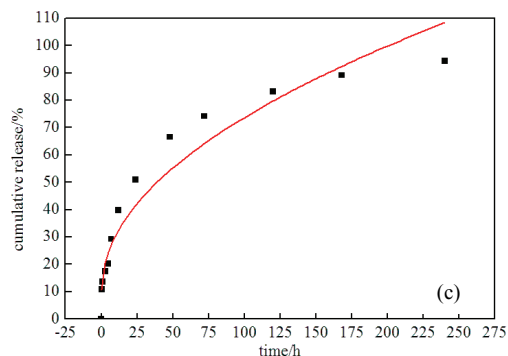
(b)

(g)

Fig.2. Cumulative Release and Fitting Curve of CS Microparticles

The goodness-of-fit of above data was also evaluated by linear regression, with values of R^2 and AIC shown in Table 4. It can be observed that R^2 values of Diffusion-Relaxation and Diffusion-Erosion were very close (0.99464, 0.99460), and slightly higher than that of First Order, Ritger-Peppas, Hixson-Crowell and Higuchi model and much higher than that of Zero Order model. However, the AIC value of Diffusion-Relaxation was much smaller (9.0933) than that of Diffusion-Erosion (13.9087), which indicates that Diffusion-Relaxation model was the best kinetic model for microparticles.

The common points of Figure 1 and Figure 2 are that the initial LOF delivery was fast and complete release of drug did not happen within test range either for nanoparticles or microparticles, which implies that the initial release might be caused by dissolution of LOF from particle surface, and those particles could not degrade or erode under detect condition within observed time. However, by comparing Figure 1 and Figure 2, it can also be concluded that the LOF release from CS microparticles was much slower than from nanoparticles.

(c)

Table 4. Fitting Equations for CS Microparticles

Models	Fitting equations	R^2	AIC
Zero Order	$F=25.19+0.37t$	0.73737	3.5885
First Order	$F=88.39（1-0.89e^{-0.03t}）$	0.97762	9.0834
Higuchi	$F=6.31t^{1/2}+10.63$	0.93341	6.6182
Ritger-Peppas	$F=19.29t^{0.31}-4.20$	0.97616	9.1752
Diffusion-Relaxation	$F=0.38+11.94\ t^{1/2}-0.39t$	0.99464	9.0933
Hixson-Crowell	$F=12.44+1.63t-0.01t^2+2.52\times10^{-5}t^3$	0.95943	23.4668
Diffusion-Erosion	$F=0.66+11.31\ t^{1/2}-0.21t-0.0015\ t^2+4.23\times10^{-6}t^3$	0.99460	13.9087

(d)

Generally speaking, drug release from matrices usually involves solvent penetration in the matrix, hydration and swelling of matrix network, diffusion of the dissolved drug, and/or the erosion of the gelatinous matrix [10]. The result demonstrated here suggests that CS microparticles prepared by emulsion crosslinking might have much tighter networks, which would result in LOF being encapsulated much hard, and the abilities, such as solvent penetration, hydration and swelling of polymer network and diffusion of LOF from interior of particles to the medium impaired obviously, and therefore much slower release rate and more prolonged release profile presented for CS microparticles.

The diverse release behavior for CS nanoparticles and microparticles determined different best fit kinetic model. However, the LOF delivery from nanoparticles or microparticles might be from surface, gap area and deep position of particles simultaneously and the mechanism of this drug release involved both drug diffusing and relaxation

(e)

(f)

process of macromolecular complex [11]. The difference of best fit kinetic model for nanoparticles and microparticles suggests that particle size might be another key physical parameter to influence LOF release from microparticles. Compared with nanoparticles, microparticles have larger diameters, and the diffusion of the dissolved LOF from interior of microparticles would suffer greater force and thus the relaxation capability of CS networks would decline as well. Therefore, LOF release from nanoparticles is dominated by diffusion process and thus abides by First Order model, while LOF release from microparticles is controlled by diffusion and relaxation process dually and therefore accords to Diffusion-Relaxation model.

Conclusions

In vitro release behaviors of CS-LOF nano/microparticles prepared by ionotropic gelatin and emulsion crosslinking technique were investigated and their release data were fitted to different mathematic models. The delivery profiles showed that the LOF release fast initially and then slowly from nano/microparticles. However, CS microparticles had more prolonged release property than nanoparticles. The best fitting kinetic models for CS nanoparticles and microparticles were First Order and Diffusion-Relaxation model respectively. The discrepancy of release mechanism between CS nanoparticles and microparticles might be related to the intensity of the polymer network and size of particles, which would be adjusted by preparing method and relative parameters.

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Authors: Corresponding author: Associate Prof. Jing Guan, Academy of Military Medical Sciences, No.106, WanDong Road, HeDong District, Tianjin, China, 300161, E-mail: janeamms@yahoo.com.cn; Co-author: Peng Cheng, Tianjin Polytechnic University, No.28, Hongxing Road, HeDong District, Tianjin, China, 300160, E-mail: badbird520@163.com；Assistant prof. Shujie Huang, Academy of Military Medical Sciences, No.106, WanDong Road, HeDong District, Tianjin, China, 300161, E-mail:shujiehuang@126.com; Dr. Zhihong Li, Academy of Military Medical Sciences, No.106, WanDong Road, HeDong District, Tianjin, China, 300161, E-mail: lizhihong1003@tom.com; Prof. Jimin Wu, Academy of Military Medical Sciences, No.106, WanDong Road, HeDong District, Tianjin, China, 300161, E-mail: whujimin07@yahoo.com.cn