Facile Preparation of Tilmicosin-Loaded Polymeric Nanoparticle with Controlled Properties and Functions

Jie Yu, Mingwei Wang,* Rizwan Ahmed, Hongyang Zhao, Martien A. Cohen Stuart, and Junyou Wang*

ABSTRACT: As one of the effective broad-spectrum antimicrobial and anti-inflammatory drugs, tilmicosin (TIM) is applied extensively in a wide range of veterinary treatments. However, the low bioavailability typically leads to overuse of TIM in practical applications, which can cause residual accumulation in the environment and contamination of foodstuffs. Here, we report a precipitation method that allows us to prepare TIM-loaded poly(methyl methacrylate-co-methacrylic acid) (P(MMA-co-MAA)) nanoparticles. Specifically, TIM and biocompatible P-(MMA-co-MAA) are dissolved in methanol and then water is introduced as an antisolvent, which triggers the co-precipitation and leads to well-controlled nanoparticles. Depending on the drug/polymer mass ratio and the total concentration of drug and polymer, the formed nanoparticles display a tunable radius from 27 to 80 nm with a narrow size distribution, a high drug loading content, and a controlled release of TIM. The encapsulation does not interrupt the antibacterial function of TIM while reducing its cytotoxicity extremely. Moreover, the formed nanoparticles could be dried to powder through freeze-drying, and the redispersion of the particles hardly disturbs the particle size, size distribution, and drug loading content. Our study developed a facile and robust precipitation method for the controlled construction of TIM-loaded polymeric nanoparticles with tunable properties and functions, as well as improved biocompatibility, which shall improve the bioavailability of TIM and enhance the practical applications.

1. INTRODUCTION

Tilmicosin (TIM) is a potent broad-spectrum antimicrobial drug and anti-inflammatory drug used for the treatment of disease caused by Gram-positive bacteria, Gram-negative bacteria, mycoplasma, spirochete, actinomyces pleuropneumonia, Pasteurella, etc. As one of the most applied anti-inflammatory drug, however, the practical application of TIM suffers a series of limitations. One is the poor water solubility of TIM due to the hydrophobic macrolide structure. Although the solubility could be improved under acid conditions, the formed TIM salt exhibits problems of low potency and low systemic bioavailability. Another limitation of TIM treatment is the acute side effects including gastrointestinal disorders, liver damage, and even cardiotoxicity at high doses. Moreover, the bitter taste of TIM influences its uptake by animals. These limitations lead to low bioavailability of TIM, and consequently, an overdose is administered to ensure the treatment effects, which causes residual accumulation in the environment and contamination of foodstuffs. Therefore, developing a new formulation that allows us to decrease the usage amount and toxicity, while keeping the treatment effects, is highly desirable.

One of the promising strategies is to load drugs in the protecting matrix, such as nanoparticles (NPs), where the encapsulation allows us to protect the drug molecules and improve solubility and biocompatibility. For example, Han et al. prepared TIM-loaded solid lipid nanoparticles through the solvent evaporation method using chloroform as a solvent and poly(vinyl alcohol) as a protecting polymer. By hot homogenization and ultrasonication method, TIM was encapsulated in hydrogenated castor oil particles, which display effective enhancement of the antibacterial activity against Streptococcus. Zhou et al. prepared TIM-loaded nanoparticles by hot melting with the ultrasonic emulsification method. In addition, TIM-loaded lipid nanocarriers prepared through the high shear method display excellent storage and gastrointestinal stability and consequently enhanced oral absorption in broilers. Zhou et al. found that TIM-loaded in alginate-choitosan nanogels shows stronger sustained drug release compared with the injection of pure TIM. Although previous studies have achieved the successful encapsulation and protection of TIM, facile preparation of TIM-loaded polymeric NPs with well-controlled size and tunable properties and functions remains a challenge.

In this paper, we demonstrate an alternative method, namely, antisolvent precipitation, for preparing TIM-loaded
NPs. Antisolvent precipitation is a facile process applied widely for the construction of drug-loaded NPs, where the solvent transfer induces the drug precipitation, which is stabilized by a protecting polymer. Here, in our work, we selected poly(methyl methacrylate-co-methacrylic acid) (P(MMA-co-MAA)) as the protecting polymer due to the approved biocompatibility of the polymer. We demonstrate that our design allows us to prepare a series of well-controlled TIM-loaded NPs with tunable properties and functions. Specifically, P(MMA-co-MAA) and TIM are first dissolved in methanol and then Milli-Q water is added dropwise, which induces the co-precipitation and formation of drug-loaded NPs. Controlling the drug/polymer ratio and the total concentration of drug and polymer allow to tune particle size, drug loading content, and release of TIM. The encapsulation does not interrupt the antibacterial function of TIM but reduces the side toxicity of TIM. Moreover, the formed NPs could be dried to prepare other drug-loaded NPs, demonstrating great potential for the design and facile preparation of drug-loaded NPs.

2. RESULTS AND DISCUSSION

The antisolvent precipitation for preparing TIM-loaded NPs is illustrated in Scheme 1a. First, both P(MMA-co-MAA) and the TIM molecule contains multiple chiral centers, and one of them is indicated by "*". TIM were dissolved in methanol in a beaker; then, Milli-Q water was added dropwise. With the addition of antisolvent (water), the TIM co-precipitated with P(MMA-co-MAA), leading to the formation of drug-loaded NPs. Below, we investigate the effects of the control factors, namely, drug/polymer mass ratio (D/P), at fixed polymer concentration) and the total concentration of drug and polymer (total D–P concentration, at fixed D/P), on the formation and controlling properties of the TIM-loaded NPs (Table S1). The formed NPs display negative surface charges (Table S2) from the carboxyl group of P(MMA-co-MAA) (Scheme 1b), which stabilize the NPs successfully as evidenced by the 4-month storage both at room temperature and 4 °C without changing the particle size and size distribution (Table S3). These results confirm that our precipitation method could prepare stable drug-loaded NPs.

2.1. Control of NP Size. The control of NP size is an important factor for the development of drug carriers. In our study, we first investigate the effects of drug/polymer mass ratio, D/P, on the particle size and size distribution. We fixed the P(MMA-co-MAA) concentration at 1.5 mg/mL and tuned the TIM concentration to achieve the designed D/P ratio at 1:1, 1:4, 1:8, 1:12, 1:24, 1:48, 1:96, and 1:192. As shown in Figure 1a, the pure P(MMA-co-MAA) forms monodispersed NPs with a hydrodynamic radius (R_h) of around 32 nm. Introducing TIM leads to the formation of bigger NPs, and the R_h increases from 39 to 60 nm with increasing the D/P ratio from 1:12 to 1:192. The NPs formed at all D/P ratios display a low PDI and narrow size distribution of the NPs.

To confirm the size increase with increasing the D/P ratio and the total D–P concentration, we characterize the NPs with TEM. As shown in Figure 2a,c, NPs prepared at the D/P ratios of 1:1, 1:2, and 1:4 are spherical NPs with diameters of 77 ± 3 nm and 119 ± 2 nm, corresponding, respectively, to the radii of 39 and 60 nm obtained from dynamic light scattering. A similar size increase was also observed in the NPs prepared at different total D–P concentrations (0.625 to 3.125 mg/mL), 54 ± 6 nm and 155 ± 3 nm), indicating that the particle size can be manipulated indeed by tuning the D/P mass ratio and the total D–P concentration. The spherical morphology of NPs was further confirmed by static light scattering, as evidenced by the angular independent diffusion coefficients of the NPs prepared at different D/P ratios and different total D–P concentrations (Figure S1).

2.2. DLC and DLE of NPs. In this section, we studied the drug loading content (DLC) and the drug loading efficiency (DLE) of the NPs prepared at different D/P ratios and different total D–P concentrations. As shown in Figure 3a, upon increasing the D/P ratio from 1:12 to 1:1 at a fixed polymer concentration of 1.5 mg/mL, the DLC increases from 7% to 32% (Figure 3a), while all samples display similar DLE of around 90% (Figure 3c). Apparently, more feeding amount of TIM is favorable for efficient encapsulation. Particularly, the 32% DLC is much higher than the values reported from the previous studies, which indicate the effective co-precipitation of TIM and P(MMA-co-MAA) based on the antisolvent precipitation. Alternatively, when we fixed the D/P ratio at 1:14, increasing the total D–P concentration from 0.625 to 3.125 mg/mL hardly changed the DLC and DLE of the formed NPs, which were about 18% and 88% (Figure 3b,d), respectively. This finding suggests that the D/P ratio is essential for the efficient encapsulation of TIM in P(MMA-co-MAA) matrix, and the total D–P concentration does not influence the DLC and DLE in our system.

2.3. TIM Release Behavior of NPs. Controlled release is crucial for designing drug-loaded NPs. Here, the release behavior of TIM-loaded NPs prepared at different D/P ratios...
and different total D−P concentrations is measured in the PBS solution at 37 °C. Figure 4a shows that NPs prepared at different D/P ratios display similar release behavior, that is, 70% of TIM is released during the first 12 h, followed by a slow release. The maximum release does not change that much upon changing the D/P ratio. However, for the case of the fixed D/P ratio with increasing total D−P concentration, the release amount of TIM decreases from 80% to 46% upon increasing the concentration from 0.625 to 3.125 mg/mL, even though the release still follows the fast–slow tendency over time (Figure 4b). It seems that the release percent decreases with the increasing particle size in this series. Increasing the size from 27 nm to 51 nm leads to a moderate decay in the release amount, while further increasing the size to 80 nm causes a sharp drop in the release percent. Moreover, the
packing density of the NPs could also be a reason for the limited release. We have characterized the NPs prepared at different total D–P concentrations by static light scattering, by which the gyration radius \( R_g \) of the NPs is determined. Then, we can estimate the packing density of the NPs based on the ratio of gyration over hydrodynamic radius \( R_g/R_h \) (Figure S2 and Table S4). It turns out that the packing density of NPs increases with the increasing particle size from 27 to 80 nm, which may explain the different release behavior.\(^{32}\) Compared with the fast release of pure TIM (Figure S3), all of the TIM-loaded NPs exhibit a slow release of TIM, demonstrating the successful encapsulation and feasibility for the controlled release of the drug.

### 2.4. In Vitro Antibacterial Activity and Cytotoxicity

The antibacterial activities were assessed using a Gram-positive bacteria *Staphylococcus aureus* (S. aureus). As shown in Table S5 and Figure S4, all of the NPs prepared at different D/P ratios and different total D–P concentrations have similar MIC (minimal inhibitory concentration) of around 4.0 \( \mu \text{g/mL} \), which is identical to the MIC of pure TIM. Next, we keep the TIM in NPs at 64 \( \mu \text{g/mL} \), then all of the formed NPs display similar inhibition zones with a diameter of around 15.6 mm (Figures 5 and S4). This finding indicates that the antibacterial activity of NPs is independent on the particle size but relies solely on the encapsulated TIM concentrations. The inhibition zone of pure TIM at the same concentration has a diameter of about 19.8 cm, based on which we conclude that the NPs retain about 80% of the antibacterial function of TIM.

To further evaluate the biocompatibility of the TIM-loaded NPs, in vitro cytotoxicity tests of NPs and free TIM against normal human cell lines hepatocyte HL7702 and lung cancer A549 cell lines were conducted. As shown in Figure 6, cell viability decreases with increasing TIM concentration both in NPs and pure TIM cases, confirming the cytotoxicity of TIM. However, at all TIM concentrations, encapsulation allows us to decrease the cytotoxicity to both HL7702 and A549 cell lines \( (p < 0.05) \). We attribute the decreased cytotoxicity to the biocompatible P(MMA-co-MAA), which is confirmed by the result that P(MMA-co-MAA) NPs without TIM hardly disrupt the viability of both HL7702 and A549 cell lines (Figure S5). In view of these findings, we conclude that P(MMA-co-MAA) indeed enhances the biocompatibility of the TIM-loaded NPs.

### 2.5. Freeze-Drying for Powder Production and Redispersibility

Freeze-drying the NP solution allows us to produce the powder, which is favorable for the storage and transport of the drug-loaded NPs.\(^{33}\) Here, in our study, we freeze-dry the solution of NPs prepared at the drug/polymer ratio of \( \frac{1}{4} \): 1, and the obtained powder is difficult to redisperse in water, indicating the possible breakage or aggregation of the NPs during the freeze-drying process. Nevertheless, the introduction of lyoprotectant or protective agent could be helpful in protecting the particles and reducing the aggregation by the formation of a glassy matrix.\(^{34}\) In this regard, we introduce glucose as a protective agent before freeze-drying and then redisperse the powder in water without changing the particle size and size distribution, as well as the drug loading content (Figure 7).

### 3. CONCLUSIONS

TIM-loaded NPs have been prepared by the antisolvent precipitation of the drug with biocompatible P(MMA-co-MAA). The design demonstrates a clear advantage in terms of controlling particle formation, properties, and functions. Specifically, increasing the drug/polymer mass ratio at a fixed polymer concentration leads to an increase in the radius of the NPs from 39 to 60 nm with a narrow size distribution, as well as an increase in drug loading content from 7 to 32%. Increasing the total concentration of drug and polymer at a fixed D/P ratio also causes the particle size to increase from 27 to 80 nm but hardly changes the drug loading content and efficiency, which are around 18 and 88%, respectively. The bigger NPs seem to have a higher packing density, and
were recorded from 30 to 150° obtained from six duplicates each with an acquisition time of processing, the average and standard deviations were obtained by a Milli-Q water purification system used in all experiments. Other reagents and solvents were used as received without any further treatment.

4.2. Characterization. The dynamic light scattering (DLS) was performed at 25 °C with an ALV-CG3 light scattering apparatus operating at a wavelength of 632.8 nm. The mean hydrodynamic radius \( R_h \) of NPs was measured at a fixed angle of 90°, while the gyration radius \( R_g \) of NPs was measured from the angular dependence of scattering, varying the angle from 60 to 150°. The CONTIN method was used to analyze the size distribution of the particle radius. For data processing, the average and standard deviations were obtained from six duplicates each with an acquisition time of 20 s. For angular-dependent DLS, 13 correlation functions were recorded from 30 to 150° at intervals of 10° to evaluate the angular dependence of the diffusion coefficient. The NP morphologies were observed on a JEOL JEM-1400 TEM instrument with an acceleration voltage of 100 kV. The NP sample solutions were diluted five times and then one drop of the diluted solution was deposited on a carbon-coated copper grid. The droplet was allowed to stay for 10 min. The UV–vis absorption spectra of the samples were recorded on a UV-1600 UV–vis spectrophotometer. The zeta-potential of NPs was characterized by Malvern Zetasizer Nano ZEN3700. Cytotoxicity was measured by absorbance at 450 nm with a Genios multifunctional reader (Tecan GENios Pro, Tecan Group Ltd., Maennedorf, Switzerland).

4.3. Preparation of NPs. The NPs were prepared by the antisolvent precipitation, as described in Scheme 1. First, designed amounts of TIM and P(MMA-co-MAA) (Table S1) were dissolved in 4 mL of methanol with stirring for 0.5 h at room temperature. To the solution, 36 mL of Milli-Q water was added dropwise under stirring for 4 h. The mixture was further stirred for 1 h at room temperature. Then, the obtained suspensions were dialyzed for 24 h against Milli-Q water (1 L of Milli-Q water per 20 mL of NP suspension, four times) using a Spectra/Por6 MWCO 10 kDa membrane to remove the remaining methanol. The final particle solutions were stored at room temperature or 4°C fridge.

4.4. Determination of DLC and DLE and the In Vitro Drug Release Study. To determine the DLC and DLE of the NPs, a calibration curve of TIM (Figure S7) was first constructed based on the UV–vis absorbance at 290 nm as a function of the TIM concentration with a UV-1600 UV–vis spectrophotometer. Then, DLC and DLE of the drug-loaded NPs were calculated as a percentage according to eqs 1 and 2, respectively

\[
\text{DLC (wt\%) = \frac{\text{amount of drug in NPs}}{\text{amount of drug loaded NPs}} \times 100} \quad (1)
\]

\[
\text{DLE (wt\%) = \frac{\text{amount of drug in NPs}}{\text{total amount of feeding drug}} \times 100} \quad (2)
\]

The release rates of TIM from the NPs were investigated with the following description. Briefly, a dialysis membrane (Spectra/Por6 MWCO 10 kDa, Spectrum Laboratories, Inc.) containing 2 mL of drug-loaded NPs was immersed in 20 mL of PBS solution (pH 7.2, 149.7 mM salt) at 37°C. At the designed time point, 1 mL of the solution outside the membrane was taken out and characterized by a UV–vis spectrophotometer at the absorption wavelength of 290 nm at 37°C.

The release UV absorbance was measured at 290 nm under different time intervals using eq 3.

\[
\text{cumulative release (\%) = } \frac{10 \times A_t}{A_0} \times 100 \quad (3)
\]

where \( A_t \) is the absorbance at time \( t \) of the outside solution and \( A_0 \) is the absorbance at the beginning 0 h of the original sample solution.

4.5. In Vitro Antibacterial Activity. The antibacterial activity test was conducted via the Kirby–Bauer method using \textit{S. aureus} bacteria. One hundred microliters of the bacterial suspension ((5–10) \times \text{10}^6 \text{ CFU/mL}) were added and grown onto nutrient agar (20 mL of the melting medium move to 9 cm culture dish). Eight millimeters of a circular filter paper was immersed into the NP solution and then placed on the surface of the agar plate and placed in an incubator at 37°C for 24 h for further analysis. Each NP sample had three duplicate tests. The diameter of the inhibition zone and the bactericidal zone for each NP sample is measured and recorded.

4.6. Cell Culture. The normal human hepatocyte HL7702 cell lines and lung cancer A549 cell lines were purchased from the Institute of Cell Biology (Shanghai, China). They were propagated in T-75 flasks cultured at 37°C under a humidified 5% CO2 atmosphere in DMEM (high glucose, Hyclone) supplemented with 10% fetal bovine serum (FBS, Biological Industry, Kibbutz Beit Haemek, Israel) and 1% penicillin–streptomycin (1 \times \text{10}^8 \text{ U/mL penicillin and 10 mg/mL streptomycin (Solarbio Life Science, Beijing, China).}

4.7. In Vitro Cytotoxicity Assay. The cell cytotoxicities of NPs toward HL7702 cell lines and A549 cell lines were measured by Cell Counting Kit-8 assay. The cell cytotoxicity was evaluated by Cell Counting Kit-8 (Dojindo, Tokyo, Japan) according to the factory’s instruction. The cells were plated in 96-well plates in 0.1 mL volume of DMEM medium with 10%
FBS and 1% penicillin–streptomycin at a density of 5 × 10^3 cells/well. Adding samples and then incubating for 48 h, we measured the absorbance at 450 nm with a Thermo Scientific microplate reader. Each sample was measured in quintuplicate. Statistical analyses were performed based on SPSS (Statistical Product and Service Solutions) software, which provides the T-test for significance and the calculation of p values.

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge at [https://pubs.acs.org/doi/10.1021/acsomega.0c04314](https://pubs.acs.org/doi/10.1021/acsomega.0c04314).

Designed amount of TIM and P(MMA-co-MAA); zeta-potential of NPs; the stability of NPs; angular-dependent light scattering results of NPs; the gyration radius \( R_g \) and \( R_h / R_g \) of the NPs; the release of pure TIM at different concentrations; MIC and MBC of TIM and TIM-loaded NPs; in vitro antibacterial activity of NPs on *S. aureus*; cytotoxicity of empty P(MMA-co-MAA) NPs; particle size and size distribution, DLC, and DLE of spiramycin-loaded NPs; and calibration curve of TIM (PDF).

**AUTHOR INFORMATION**

**Corresponding Authors**

Mingwei Wang — Shanghai Key Laboratory of Multiphase Materials Chemical Engineering, School of Chemical Engineering, East China University of Science and Technology, Shanghai 200237, People’s Republic of China; orcid.org/0000-0001-7071-9166; Email: mingweiwang@ecust.edu.cn

Junyou Wang — Shanghai Key Laboratory of Multiphase Materials Chemical Engineering, School of Chemical Engineering, East China University of Science and Technology, Shanghai 200237, People’s Republic of China; orcid.org/0000-0002-4693-850X; Email: junyouwang@ecust.edu.cn

**Authors**

Jie Yu — Shanghai Key Laboratory of Multiphase Materials Chemical Engineering, School of Chemical Engineering, East China University of Science and Technology, Shanghai 200237, People’s Republic of China

Rizwan Ahmed — Shanghai Key Laboratory of Multiphase Materials Chemical Engineering, School of Chemical Engineering, East China University of Science and Technology, Shanghai 200237, People’s Republic of China

Hongyang Zhao — Shanghai Key Laboratory of Multiphase Materials Chemical Engineering, School of Chemical Engineering, East China University of Science and Technology, Shanghai 200237, People’s Republic of China

Martien A. Cohen Stuart — Shanghai Key Laboratory of Multiphase Materials Chemical Engineering, School of Chemical Engineering, East China University of Science and Technology, Shanghai 200237, People’s Republic of China

Complete contact information is available at: [https://pubs.acs.org/10.1021/acsomega.0c04314](https://pubs.acs.org/10.1021/acsomega.0c04314)

**Notes**

The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

This work was supported by the “1000 Foreign Experts Program” (WQ20163100341), the National Natural Science Foundation of China (NSFC) for Young Scholars (21706074), and the Fundamental Research Funds for the Central Universities (222201814007).

**REFERENCES**

(1) Zhang, P.; Hao, H. H.; Li, J.; Ahmad, I.; Cheng, G. Y.; Chen, D. M.; Tao, Y. F.; Huang, L. L.; Wang, Y. L.; Dai, M. H.; Liu, Z. L.; Yuan, Z. H. The Epidemiologic and Pharmacodynamic Cutoff Values of Tilmicosin against *Haemophilus parasuis*. *Front. Microbiol.* 2016, 7, 143.

(2) Yudthavorasit, S.; Chiaoachan, C.; Leepipatpiboon, N. Simultaneous determination of multi-class antibiotic residues in water using carrier-mediated hollow-fiber liquid-phase microextraction coupled with ultra-high performance liquid chromatography tandem mass spectrometry. *Microchim. Acta* 2011, 172, 39–49.

(3) Zhang, Q.; Yang, H.; Sahito, B.; Li, X.; Peng, L.; Gao, X.; Ji, H.; Wang, L.; Jiang, S.; Guo, D. Nanostructured lipid carriers with exceptional gastrointestinal stability and inhibition of P-gp efflux for improved oral delivery of tilmicosin. *Colloids Surf., B* 2020, 187, No. 110649.

(4) Sahito, B.; Zhang, Q.; Yang, H.; Peng, L.; Gao, X.; Kashif, J.; Ul Aabdin, Z.; Jiang, S.; Wang, L.; Guo, D. Synthesis of Tilmicosin Nanostructured Lipid Carriers for Improved Oral Delivery in Broilers: Physiochemical Characterization and Cellular Permeation. *Molecules* 2020, 25, 315.

(5) Zhou, K.; Wang, X.; Chen, D.; Yuan, Y.; Wang, S.; Li, C.; Yan, Y.; Liu, Q.; Shao, L.; Huang, L.; Yuan, Z.; Xie, S. Enhanced Treatment Effects of Tilmicosin Against Staphylococcus aureus Cow Mastitis by Self-Assembly Sodium Alginate-Chitosan Nanogel. *Pharmaceutics* 2019, 11, 524.

(6) Wang, Q.; Pei, X. Y.; Peng, T.; Xie, J.; Xie, S. L.; Sun, Y. Z.; Wang, C.; Li, X. M.; Jiang, H. Y. Determination of Tilmicosin by Fluorescence-Based Immunochromatography. *Anal. Lett. 2016*, 49, 2052–2062.

(7) Dong, Z.; Zhou, X.; Sun, J.; Meng, X.; Li, H.; Cheng, F.; Wei, X.; Li, B.; Wang, W.; Zhang, J. Efficacy of enteric-coated tilmicosin granules in pigs artificially infected with Actinobacillus pleuropneumoniae serotype 2. *Vet. Med. Sci.* 2020, 6, 105–113.

(8) Yang, Y.; Yuan, L.; Li, J.; Muhammad, I.; Cheng, P.; Xiao, T.; Zhang, X. Preparation and evaluation of tilmicosin microspheres and lung-targeting studies in rabbits. *Vet. J.* 2019, 246, 27–34.

(9) Li, X.; Wu, X.; Wang, J.; Hua, Q.; Wu, J.; Shen, X.; Sun, Y.; Lei, H. Three lateral flow immunochromatographic assays based on different nanoparticle probes for on-site detection of tylosin and tilmicosin in milk and pork. *Sens. Actuators, B* 2019, 301, No. 127059.

(10) Li, X.; Shen, J.; Wang, Q.; Gao, S.; Pei, X.; Jiang, H.; Wen, K. Multi-residue fluorescent microspheres immunochromatographic assay for simultaneous determination of macrolides in raw milk. *Anal. Bioanal. Chem. 2015*, 407, 9125–9133.

(11) Li, Y.; Li, W.; Bao, W.; Liu, B.; Li, D.; Jiang, Y.; Wei, W.; Ren, F. Bioinspired peptosomes with programmed stimuli-responses for sequential drug release and high-performance anticancer therapy. *Nanoscale* 2017, 9, 9317–9324.

(12) Teng, W.; Zhao, L.; Yang, S.; Zhang, C.; Liu, M.; Luo, J.; Jin, J.; Zhang, M.; Bao, C.; Li, D.; Xiong, W.; Li, Y.; Ren, F. The hepatic-targeted, resveratrol loaded nanoparticles for relief of high fat diet-induced nonalcoholic fatty liver disease. *J. Controlled Release* 2019, 307, 139–149.

(13) Zhu, Z. Flash Nanoprecipitation: Prediction and Enhancement of Particle Stability via Drug Structure. *Molecules* 2014, 11, 776–786.

(14) Chen, W.; Yung, B. C.; Qian, Z.; Chen, X. Improving long-term subcutaneous drug delivery by regulating material-bioenvironment interaction. *Adv. Drug Delivery Rev.* 2018, 127, 20–34.
An Essential Material in Medicine and Dentistry. Pharm. 2020

dispersions containing darunavir by coaxial electrospraying. Int. J.
Mooter, G. V. D. Gastro-resistant encapsulation of amorphous solid
Med. Implants 2005

copolymer for GRAS evaluation. Regul. Toxicol. Pharmacol.

Copolymerization and toxicological assessment of Neutral Methacrylate
poly(−caprolactone) nanoparticles. Carbohydr. Polym. 2013

Wang, X. F.; Zhang, S. L.; Zhu, L. Y.; Xie, S. Y.; Dong, Z.; Wang, Y.; Zhou, W. Z. Enhancement of antibacterial activity of tilmicosin against Staphylococcus aureus by solid lipid nanoparticles in vitro and in vivo. Vet. J. 2012, 191, 115–120.

Zhou, K. X.; Yan, Y. Y.; Chen, D. M.; Huang, L. L.; Li, C.; Meng, K. Y.; Wang, S. G.; Algharib, S. A.; Yuan, Z. H.; Xie, S. Y. Solid Lipid Nanoparticles for Duodenum Targeted Oral Delivery of Tilmicosin. Pharmaceutics 2020, 12, 731.

Li, B.; Wang, Q.; Wang, X.; Wang, C.; Jiang, X. Preparation, drug release and cellular uptake of doxorubicin-loaded dextran-b-poly(ε-caprolactone) nanoparticles. Carbohydr. Polym. 2013, 93, 430–437.

Xie, J.; Lee, S.; Chen, X. Nanoparticle-based theranostic agents. Adv. Drug Delivery Res. 2010, 62, 1064–1079.

Frazier, R. Q.; Byron, R. T.; Osborne, P. B.; West, K. P. PMMA: An Essential Material in Medicine and Dentistry. J. Long-Term Eff. Med.Implants 2005, 15, 629.

Rahul, B.; Bhola, S. M.; Liang, H.; Mishra, B. Biocompatible denture polymers - A review. Trends Biomater. Artif. Organs 2010, 23, 129–136.

Smeets, A.; Koekoekx, R.; Ruelens, W.; Smet, M.; Clasen, C.; Mooter, G. V. D. Gastro-resistant encapsulation of amorphous solid dispersions containing darunavir by coaxial electrospraying. Int. J. Pharm. 2020, 574, No. 118885.

Eisele, J.; Haynes, G.; Kreuzer, K.; Rosamilia, T. Characterisation and toxicological assessment of Neutral Methacrylate Copolymer for GRAS evaluation. Regul. Toxicol. Pharmacol. 2013, 67, 392–408.

Hirot, T. Orally Disintegrating Tablet Useful for Treating Alzheimer’s Disease, Comprises Drug Contained in Granule Comprising Water-insoluble Polymer and Enteric Polymer. Patent WO2019098327A1, Nov 17, 2017.

Wang, M.; Lin, S.; Wang, J.; Liu, L.; Zhou, W.; Ahmed, R. B.; Hu, A.; Guo, X.; Cohen Stuart, M. A. Controlling Morphology and Release Behavior of Sorafenib-Loaded Nanocarriers Prepared by Flash Nanoprecipitation. Ind. Eng. Chem. Res. 2018, 57, 11911–11919.

Ghaffari, S.; Varshosaz, J.; Saadat, A.; Attyabi, F. Stability and antimicrobial effect of amikacin-loaded solid lipid nanoparticles. Int. J. Nanomed. 2011, 6, 35–43.

Fonte, P.; Araújo, F.; Seabra, V.; Reis, S.; van de Weert, M.; Sarmento, B. Co-encapsulation of lyoprotectants improves the stability of protein-loaded PLGA nanoparticles upon lyophilization. Int. J. Pharm. 2015, 496, 850–862.