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Eco-Friendly Spiking Approach Based on Microfluidics for Preparation of Matrix Reference Materials

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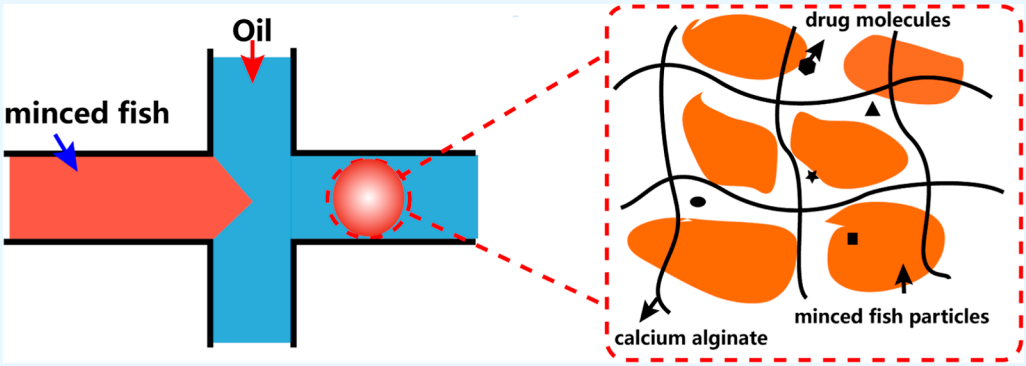

ABSTRACT: Medicated bath is the most common spiking method used in the development of matrix reference materials for aquatic products; however, the environmental issues caused by the treatment of waste liquid after medicated bath cannot be ignored. We proposed an environmentally friendly spiking method based on microfluidics, which significantly improved the drug utilization rate without the need for subsequent drug residue treatment. Finely processed minced fish samples were fully mixed with quinolone drugs, and minced fish gel microspheres were prepared by microfluidic technology, utilizing the gel's water-locking function to enhance the drug-loading capacity. The results showed that this method can significantly increase the drug-loading capacity of the matrix (2.33−4.03 times) compared with the traditional spiking methods. In addition, the matrix reference material prepared by this method has good stability, and the drug concentration was adjustable and controllable.

1. INTRODUCTION

In recent years, food safety has attracted significant attention.[1] An urgent issue that needs to be addressed is how to rapidly and accurately detect drug residues.[2] Reference materials play a crucial role in quality control for the detection and analysis of drug residues.[3] These materials possess sufficient homogeneity and stability, providing the prerequisite and foundation for ensuring the accuracy of analytical testing.[4] In the field of aquatic product analysis, medicated bath is often used to prepare matrix reference materials.[5] This method involves immersing the sample in a solution containing specific chemicals to simulate or introduce target analytes into the sample matrix. While this method plays a key role in ensuring the accuracy and reliability of analytical results, its potential environmental impacts cannot be ignored.

After completion of the soaking process, the waste liquid generated contains drug residue and other potentially harmful chemical components, leading to three primary environmental impacts. First, water pollution.[6,7] Drug residues in the water are difficult to degrade, and their accumulation may lead to excessive levels in the water body, disrupting ecological balance and affecting marine biodiversity. Second, soil pollution.[8] When the soaking sample's waste liquid is discharged into irrigated fields or seeps into the soil without treatment, drug compounds may deposit, alter the microbial community structure in the soil, reduce soil fertility, and potentially enter the ecosystem through food chains. Third, increased antibiotic resistance.[9] Frequent use of soaking methods may make cultured organisms resistant to drugs, which increases the required dosage and increases the likelihood of environmental drug residue accumulation. In addition, drug resistance may be transmitted to nontarget organisms such as wild aquatic animals or microorganisms, exacerbating the spread and proliferation of drug resistance globally.

Faced with these environmental challenges, researchers have developed numerous effective methods for treating waste solutions.[10] Presently, physical−chemical treatment techniques include adsorption, precipitation/flocculation, reverse osmosis,

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and ultrafiltration.[11−13] Adsorption can effectively remove drug residuals in waste solutions using activated carbon, molecular sieves, and other materials followed by desorption and recovery.[14−16] Precipitation/flocculation achieves separation through the addition of flocculants to form insoluble drug ions.[17] Reverse osmosis and ultrafiltration technologies utilize semipermeable membranes to filter out drug residuals and other contaminants. Additionally, biological treatment technologies like microbial degradation and biofilm techniques have also gained considerable interest, especially where highly efficient degrading microorganisms or those found on biofilms transform and degrade drug residuals biologically.[18] Despite these methods' effectiveness in significantly lowering drug residuals and other hazardous substances in soak solutions, they often face drawbacks such as high costs, complex processes, and difficulties with regeneration and energy consumption.[19] Physical−chemical treatments require expensive adsorbent materials and chemical reagents, while biological treatments might have slow degradation rates, stringent conditions, and long processing times along with costly research and development to screen and cultivate highly efficient degrading strains.[20]

Considering the economic investment required for postsoak processing, exploring alternative methods to the soaking technique might represent a new attempt at addressing pollution from its source. In this regard, we propose an innovative approach based on microfluidic technology for preparing matrix standard materials. This method fully leverages the advantages of thorough mixing between liquids to ensure that drug solutions can mix completely with blank matrices. Moreover, it employs hydrogel-based moisture retention principles to improve the utilization rate of the drug solution, producing minimal waste. Critically, it enables precise regulation of the drug content in matrix standard materials by adjusting the initial concentration of the drug solution, thus conserving drugs and adhering to green chemistry principles. Matrix standard materials prepared by using this method exhibit excellent stability and present a promising new direction for consideration.

2. EXPERIMENTAL SECTION

2.1. Materials and Instruments. The National Institute of Metrology provided enrofloxacin (GBW10155) and ciprofloxacin (GBW10156). Norfloxacin (GBW09251) was acquired from the Institute of Quality Standard and Testing Technology of CAAS. Solarbio (Shenzhen Selma Biotechnology Co.) supplied sodium alginate (SA). Additionally, the local aquatic market supplied fresh grass carp, with an average weight of approximately 1 kg. The minced fish samples were obtained using a high-speed homogenizer (GD-16, ShenZhen XinRui Technology Development Co., Ltd., China). Tandem mass spectrometry (HPLC-MS, QTRAP-5500, SCIE, USA) was employed for detection using high-performance liquid chromatography. All samples were freeze-dried with a large freeze-dryer (5265, ZIRBUS, Germany). A multifunctional grinder (BJ-1000A, Baijie, China) is used for sample crushing.

2.2. Preparation of Microfluidic Chips. A microfluidic chip was created by using soft lithography. The SU-8 2050 photoresist was uniformly coated with spin on a 75 mm silicon wafer at a speed of 1250 rpm. The spin-coating procedure had a duration of 90 s and a thickness of 140 μm. After a delicate baking procedure at 70 °C for 5 min, the silicon wafer underwent patterning by applying ultraviolet exposure with a transparent photomask. Afterward, the baking process was repeated at a temperature of 75 °C for 20 min and the development procedure was continued using SU-8 developer. After the microstructure was completed, the mold was subjected to silanization reagents (1*H*,1*H*,2*H*,2*H*-perfluorooctyltrichlorosilane) in vacuum for 6 h. A mixture of degassed PDMS and initiator 10:1 was poured into the mold and allowed to cure at 65 °C for 3 h. After the PDMS chip was cured, it is taken out of the mold and precisely punched (inlet and outlet). The PDMS chip was securely affixed to the glass substrate after exposure to oxygen-containing plasma on the surface.

2.3. Sample Preparation. The muscle tissue of fresh grass carp was selected and preliminarily treated with a meat grinder to form meat particles. 5 g of the above-mentioned meat samples and 15 steel balls are weighed into a 50 mL centrifuge tube (10 pieces of 8 mm, 5 pieces of 5 mm) and are ground with a high-speed homogenizer. The grinding is divided into three stages: the first stage is at 1500 rpm for 1 min, the second stage grinding time is 4 min at 2000 rpm, and the third stage grinding for 2 min at 2500 rpm. The ground minced fish samples were collected and filtered using a 300-mesh metal sieve to remove the membrane-like tissue that was not crushed in the sample. The filtered sample was centrifuged, and the supernatant fluid was removed. Then, the minced fish sample was dissolved in distilled water to prepare a minced fish solution in a ratio of 1:1. Subsequently, three quinolone drug solutions of 5, 10, 15, and 25 μg/kg were prepared. The above solution was fully mixed with the prepared minced fish solution in a ratio of 1:1 to ensure that the minced fish particles fully absorbed the drug molecules. Finally, a 1% SA solution was prepared and mixed with the above solution in a ratio of 1:1 to create a mixed solution of minced fish, drugs, and SA. This mixed solution was then microfluidically processed into minced fish gel microspheres using a microfluidic fluid shearing device; the minced fish gel microspheres used in this work are as shown in Figure S1. The minced fish gel microspheres were washed and freeze-dried by using a large freeze-drying machine.

Water was injected into three bathtubs measuring 97 × 57 × 60 cm each, with a height of approximately 27 cm. Each tank contained three grass carp weighing 1.5 ± 0.2 kg. The concentration of the mother liquor of the three quinolones was 2 g/L, and 29.75 mL of the drug solution was added to each bathtub. The dosing time interval between the first drug and the third drug should not exceed 20 min. The process was conducted at room temperature (25 ± 0.5 °C) for 8 h. Muscle tissue from the grass carp after the medicated bath was selected and ground following the operational steps outlined in the previous paragraph. The ground sample solution was filtered and centrifuged, and 1 g of the centrifugal precipitate was weighed for drug extraction.

Healthy and fresh negative grass carp samples were selected, and the muscle tissues on both sides were taken after slaughter. The evenly mixed fish meat was evenly tiled on the tray, and the thickness was controlled below 0.5 cm. The prefreezing temperature was set according to the eutectic temperature of the freeze-dried material. The prefreezing temperature of the fish was set to −20 °C, and the setting time was 60 min (cooling to −20 °C within 60 min). After 60 min of maintenance, the prefreezing effect of the fish reached the best; the setting temperature of the water catcher is 50 °C below zero, and the duration is 20 min. The prepumping

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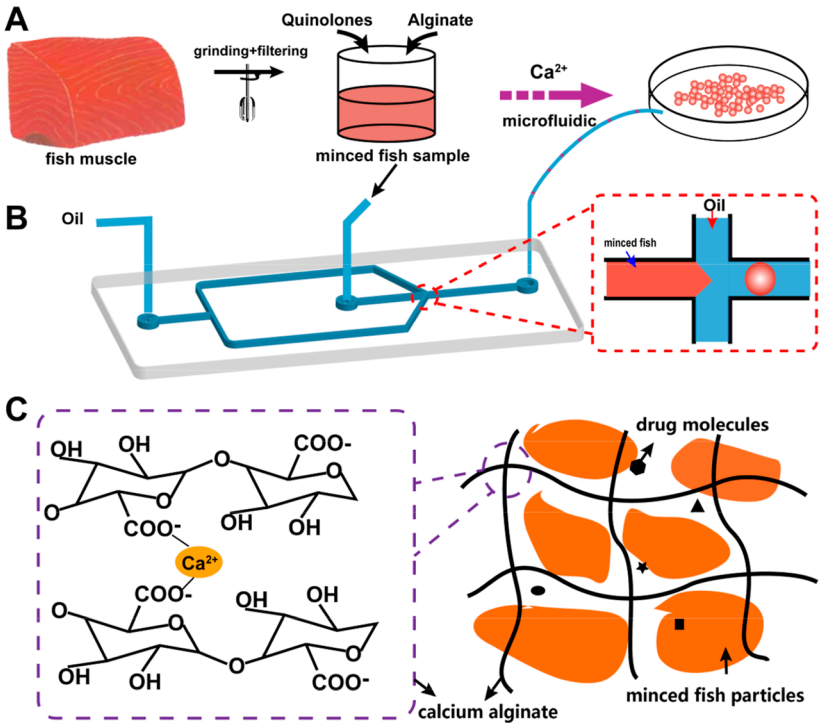

Figure 1. Schematic of the experimental mechanism. (A) Flowchart of sample preparation and processing of minced fish samples; (B) schematic diagram of the microfluidic chip device and fish gel microsphere preparation; (C) adsorption of drug molecules on minced fish samples.

vacuum is set to 0.2 Mbar, and the alarm vacuum is set to 0.35 Mbar. The first drying was divided into two stages. In the first stage, the temperature was set to 0 °C, and the setting time was 510 min (the temperature was increased from −20 to 0 °C within 510 min). After 180 min, the vacuum degree was maintained at 0.2 Mbar. In the second stage, the temperature was set to 40 °C, the setting time was 330 min (from 0 to 40 °C within 330 min), and the vacuum degree was maintained at 0.2 Mbar; the desorption drying temperature was set to 45 °C, the setting time was 90 min (from 40 to 45 °C within 90 min), and the vacuum degree was maintained at 0.1 Mbar after 120 min. The temperature of the product is maintained at about 45 °C, the temperature difference is within 5 °C, and the freeze-drying operation can be shut down.

The freeze-dried grass carp muscle tissue was crushed and ground by a crusher for 30 min to make a freeze-dried fishmeal. The freeze-dried fishmeal was sieved with 120 mesh sieves. 200 × 4 g of freeze-dried fish meal was evenly spread on three clean trays, and three kinds of quinolone solutions with different concentrations prepared before were sprayed on the surface of fish meal. They were stirred well to fully mix the drug solution with the freeze-dried fish meal. Then it was put into a large freeze-dryer and freeze-dried again. 1 g of freeze-dried fish meal containing drugs for drug extraction was weighed.

2.4. Extraction Method. A 50 mL sealed lid centrifuge tube was used to precisely weigh 1 g of freeze-dried samples, followed by the addition of 4 mL of ultrapure water and vigorous agitation at 2500 rpm for 10 min. 50 μL of internal standard working fluid was incorporated with precision and agitated manually for 30 s. Additionally, a mixture of 15 mL of acetonitrile and one percent formic acid was included. The sample was vigorously stirred for 5 min; then 4 g of anhydrous magnesium sulfate was added to remove any surplus water. Once the sample had been completely mixed, it was subjected to a 10 min ultrasonic extraction process to separate the seven types of quinolones from the sample completely. The sample was centrifuged at a rate of 4000 rpm for a duration of 10 min. The liquid was placed in a 50 mL sealed lid centrifuge tube, and the process was repeated once. Once the two supernatants were evenly blended, 15 mL of nitrogen was gathered and subsequently desiccated. After blowing nitrogen, the sample was mixed with 1.0 mL of 20% methanol aqueous solution and 3 mL of *n*-hexane, stirred for 1 min, evenly mixed, and then spun in a centrifuge at 12,000 rpm for 5 min. A 1 mL syringe was employed to take in the lower complex solution, which was then passed through a 0.22 μm filter membrane.

2.5. Establishment of the MS Method. The prepared samples were evaluated by using liquid chromatography−tandem mass spectrometry (LC−MS/MS). The determination of the chromatographic conditions involved the utilization of a liquid chromatographic column (100 × 2.1 mm, 5 μm), a column temperature of 40 °C, an injection volume of 5 μL, a flow rate of 0.3 mL/min, and a mobile phase comprising 0.002 mol/L ammonium acetate solution and methanol. The mass spectrometry reference conditions consist of an electrospray ion source (ESI) in positive ion mode, a spray voltage (IS) of 5500 V, an ion source temperature (TEM) of 650 °C, a collision gas (CAD) of medium, a curtain gas (CUR) of 30 psi, an atomizer (GS1) of 60 psi, an auxiliary heating gas (GS2) of 55 psi, a DP of 80 V, an EP of 10 V, a collision chamber output voltage (CXP) of 10 V, and a scanning mode utilizing multiple reaction monitoring.

The mixed standard working solution of 10 and 100 ng/mL was accurately measured and then configured into a gradient solution with a concentration of 0.5, 1, 2, 5, 10, and 20 ng/mL. According to the operation steps of Section 2.4, the extraction was carried out. The standard curve was drawn with the solution concentration as the abscissa and the ratio of the characteristic ion peak area of the external standard to the

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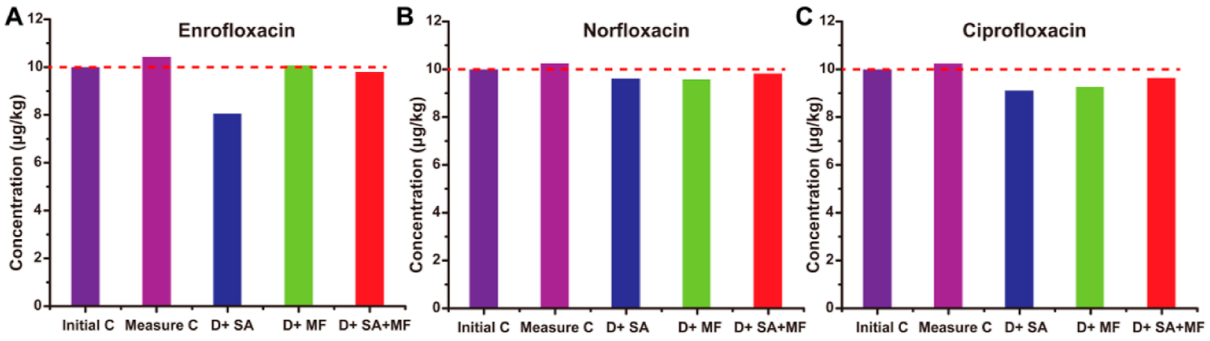

Figure 2. Comparison of the effects of different factors on the three quinolones. A, B, and C represent enrofloxacin, norfloxacin, and ciprofloxacin, respectively. The symbols initial C, measure C, D, SA, and MF in the figure represent the initial drug concentration, the measured drug concentration of the pure solution, the drug solution, the SA solution, and the minced fish sample.

internal standard as the ordinate, and the actual measured concentration was calculated according to the standard curve.

2.6. Statistical Analysis. To ensure the accuracy of the results, all of the experiments were repeated at least three times. The error caused by human factors in the process of three different labeling methods was avoided through multiple experiments. All mass spectrum values are obtained from the average of at least three measurements. All possible numerical deviations were experimentally verified.

3. RESULTS AND DISCUSSION

3.1. Experimental Design and Experimental Principle. Medicated baths are the most commonly used spiking methods for preparing matrix standard materials in aquatic products. The negative sample was immersed in the drug solution to carry the target drug by adsorption or absorption. This approach allows for the simultaneous processing of many samples. However, the drug content in the matrix material obtained through this method is uncontrollable since the metabolic rate of organisms can be influenced by external environmental factors. The uneven distribution or loss of drugs during the subsequent sample processing steps, such as washing, drying, and grinding, could occur due to human errors or operational inconsistencies. The accuracy and reliability of the matrix reference material prepared by using the medicated bath method may be compromised. Moreover, the treatment of the soaked solution after the medicated bath typically requires a series of complex steps and rigorous operating procedures. This is because the soaked solution contains drug residues and other potentially harmful substances, which can cause environmental problems. Therefore, it is necessary to explore more precise and controllable spiking methods to improve the overall accuracy and solve environmental pollution problems.

Herein, we propose a new spiking method based on microfluidics, as shown in Figure 1. There are two advantages by employing microfluidic techniques to generate minced fish gel microspheres from well-treated fresh muscle tissue. One is to make full use of the characteristics that liquid and liquid can be fully mixed to ensure that minced samples can fully absorb drugs. The second is to use the water-locking characteristics of the gel to encapsulate the drug molecules in the gel microspheres to prevent drug loss. To our knowledge, there is almost no precedent for utilizing microfluidic chips in the processing of fish mince samples.

Differing from conventional liquids, fish paste samples tend to cause clogging in microfluidic chip channels. Consequently, as depicted in Figure 1A, we conducted refined processing on muscle tissues. This involved grinding grass carp muscle tissue using a high-speed homogenizer, followed by the removal of membrane-like tissues mixed within via a metal filter. Following the centrifugation process to eliminate the supernatant from the filtered sample, it was then mixed with deionized water in a ratio of 1:1 to prepare a minced sample solution. Subsequently, a preprepared quinolone solution was added to the minced sample solution, which was thoroughly mixed by stirring, and then mixed with 1% SA solution in a ratio of 1:1. Finally, the minced fish gel microspheres were prepared by fluid shear using a microfluidic chip, as shown in Figure 1B. Utilizing the interface between the oil and water phases, fluorinated oil was used to ensure that the drug was not lost. As shown in Figure 1C, when the SA solution encounters calcium ions, a cross-linking reaction occurs, forming a dense network structure. This structure encapsulates fish paste particles and drug molecules, further minimizing the loss of the drug. The drug utilization rate is almost 100%, and therefore, there is almost no risk of environmental pollution caused by drug loss.

3.2. Validation of the Matrix Effect. Generally, during the production process of matrix reference materials, it is crucial to ensure that the matrix composition of the reference material is consistent with or as close as possible to that of the actual sample. In this method, a small amount of SA was introduced as a fixative and whether it influenced the drug or matrix was the key to the success of the method. To validate the effects of SA on the matrix and the drugs, comparative experiments were carried out as follows. First, the standard curves of three quinolones were obtained by high-performance liquid chromatography−mass spectrometry (HPLC−MS), and the test results are shown in Figure S2. Then, the pure solution with a concentration of 10 μg/kg was detected, respectively, and the test results were obtained according to the standard curve to verify the stability of the drug itself. Second, the above-mentioned 10 μg/kg standard solution was mixed with 1% SA solution, and the actual results were detected after mixing with calcium oxalate solution to determine whether SA would bind with drug molecules. Subsequently, the 10 μg/kg solution was fully mixed with the same amount of fish meat matrix to detect its concentration, which was used to assess the effect of the fish meat matrix on the drug. Finally, the 10 μg/kg drug solution was thoroughly mixed with the SA solution and then added to a minced fish solution. After being fully mixed, it was mixed with the calcium oxalate solution to form a minced

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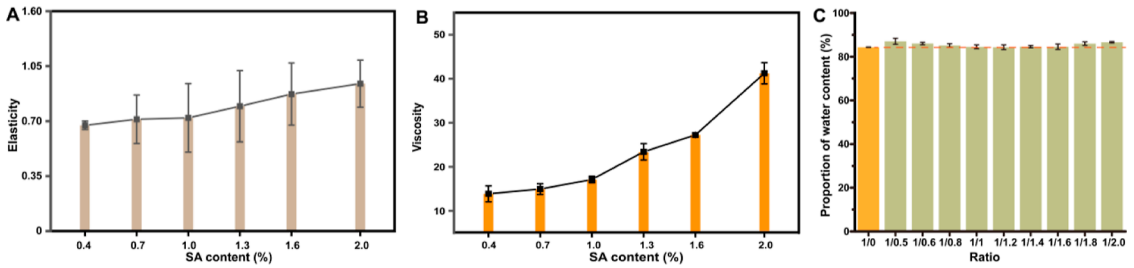

Figure 3. Optimization of the SA content. (A–C) Corresponds to the elasticity, viscosity, and water content of minced fish gel microspheres prepared with different contents of SA.

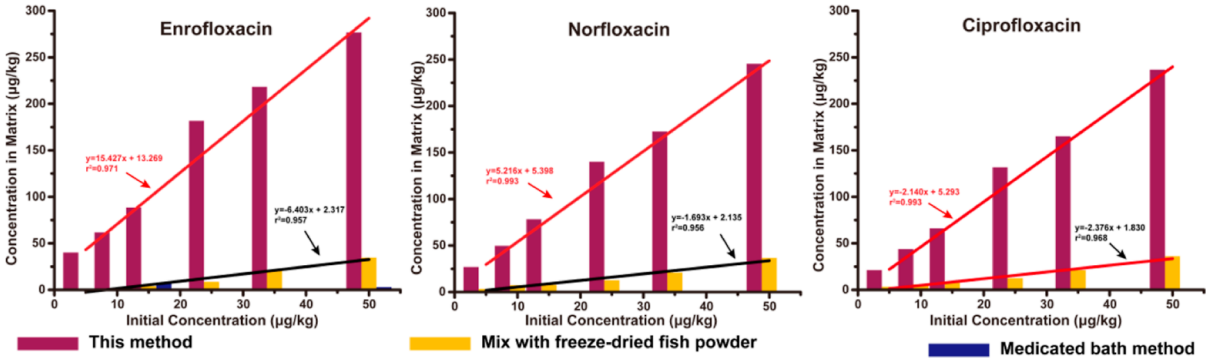

Figure 4. Comparison of three quinolone-loading capacities by three different spiking methods. Red represents this method, yellow represents the spraying method, and blue represents the medicated bath method. All the experimental data in this figure are represented by the average of the three experiments.

fish gel, and the drug concentration was measured. The results are as shown in Figure 2.

In Figure 2A, the initial concentration of enrofloxacin was 10 μg/kg, and the test result was 10.43 μg/kg, which was basically consistent with the initial result, indicating that enrofloxacin remained stable during the experiment. When the enrofloxacin solution was mixed with 1% SA solution to prepare SA gel, the detection result was 8.06 μg/kg, which means that SA or calcium ions may bind to enrofloxacin. At the same time, when the drug solution was only mixed with the minced fish sample, the detection result was about 10.07 μg/kg, which indicated that the protein components or other components in the minced fish sample were not combined with the drug and the matrix effect can be ignored. Finally, the mixed solution containing enrofloxacin, SA, minced fish sample, and calcium oxalate was tested, and the result was about 9.79 μg/kg, indicating that SA has few effects on enrofloxacin. In Figure 2B, the initial concentration of norfloxacin started at 10 μg/kg, and the subsequent testing revealed a value of 10.25 μg/kg, showing a close correspondence to the original reading and thus demonstrating the stability of norfloxacin during the experimental process. Upon mixing the norfloxacin solution with a 1% SA solution to create a SA gel, the detected concentration came out to be 9.62 μg/kg, signifying that SA or calcium ions did not bind to norfloxacin, leading to no loss of the drug. Concurrently, when the drug solution was solely combined with the minced fish sample, the measurement registered around 9.58 μg/kg, indicating the interaction between the protein components or other elements present in the minced fish sample and the drug. Lastly, a mixture of norfloxacin, SA, minced fish, and calcium oxalate was analyzed, and the outcome was approximately 9.82 μg/kg, reiterating that SA exerted no impact on norfloxacin. In Figure 2C, the initial concentration of ciprofloxacin was 10 μg/kg, and subsequent measurements resulted in a value of 10.24 μg/kg, closely matching the initial value and verifying the chemical stability of ciprofloxacin throughout the experimental procedure. Following the combination of the ciprofloxacin solution with a 1% SA solution to form an alginate gel, the recovery rate is 91.1% (9.11 μg/kg), demonstrating that SA or calcium ions did not bind to the antibiotic, ensuring that no loss of the drug occurred. Simultaneously, when the drug solution was mixed only with the fish homogenate sample, the detected concentration was approximately 9.25 μg/kg, indicating that the proteins and other constituents present in the minced fish sample interacted with the drug. Finally, after examining the blend of ciprofloxacin, SA, minced fish, and calcium oxalate, we found that the measured outcome was roughly 9.64 μg/kg, reinforcing the notion that SA did not affect the properties of ciprofloxacin in any way.

A low-concentration SA solution at such levels will not impact the minced fish-based matrix. For the rigor of the experiment and to ensure that the matrix reference material prepared by this method resembled or maintained consistency with the attributes of the actual samples, comparative assessments of elasticity, viscosity, and water content were carried out. The experimental conditions were further optimized; the experimental results are shown in Figure 3. The results showed that the content of SA affects the physical properties of fish mince gel microspheres. From Figure 3A,B, it can be observed that with the increase in SA content, the elasticity and viscosity of fish mince gel microspheres also increase continuously, especially when the SA content reaches 1%, the change trend becomes more significant. In addition, the water content of fish mince gel microspheres (Figure 3C) also changes with the variation in SA concentration. When the SA concentration is 1%, its water content is basically consistent with that of fresh fish meat samples; therefore, a concentration

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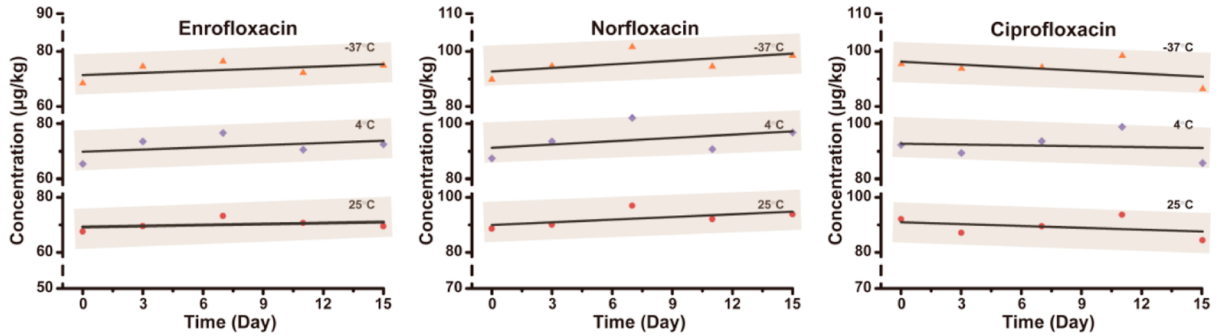

Figure 5. Tendency chart of the short-term stability test results of three quinolones at −37, 4, and 25 °C. The experimental data in this figure are the average of five measurements.

of 1% SA solution was ultimately chosen. When a 1% SA solution is mixed with the drug solution in a 1:1 ratio, the actual concentration of SA is less than 0.5%. Hence, the introduction of SA in this method does not have an adverse effect on the matrix.

3.3. Comparison of Different Methods. To verify the effectiveness of this method, three spiking methods were compared: this method and two traditional spiking methods, namely, the medicated bath method and spraying method. The solution of enrofloxacin, norfloxacin, and ciprofloxacin with different concentrations (5, 10, 15, 25, 35, and 50 μg/kg) was prepared. Fresh grass carp was the research object, which contained no quinolone residues. The experiments were carried out in strict accordance with the experimental conditions of Section 2.2. Qualitative and quantitative detection and analysis of three samples were carried out by HPLC−MS. The results are shown in Figure 4.

Two conclusions were obtained from the above results: first, this method and spray labeling method are suitable for the development of matrix reference materials containing multiple drugs, in which the drug-loading capacity has a linear correlation with the initial drug concentration. The linear correlation coefficients of three quinolones of these two methods are 0.971, 0.993, and 0.993 and 0.957, 0.956, and 0.968, respectively. Although there are some differences between these two methods, both meet the needs of the matrix reference material which contains multidrugs. However, the medicated bath method resulted in huge differences in the drug-loading capacity of different drugs and linear irrelevance.

Second, the drug-loading regulation effect of this method is much better than the traditional spiking methods, in which the same initial drug concentration can obtain a better drug-loading effect. Table S1 shows the content of drugs in the matrix reference material prepared by the three methods with different initial concentrations. The introduction of the spraying method was to compare the water-locking effect of SA. The results indicated that SA can efficiently lock drug molecules, thereby significantly enhancing its drug-loading capacity. The drug concentration in the matrix reference material prepared by this method was 2.33−4.03 times the initial concentration, while the drug content in the other two methods was much lower than the initial drug concentration. This means that this method can obtain a better regulation effect with the same initial concentration conditions. More importantly, matrix reference materials with high drug concentrations can be obtained by using a low-concentration initial drug solution, which avoids environmental pollution and conforms to the concept of green development.

3.4. Stability of the Matrix Reference Material. According to the requirements of "JJF 1343-2012: general principle of certified reference materials and statistical principle", the short-term stability investigation of certified reference materials mainly evaluates the change or influence of the characteristic value of certified reference materials during transportation due to the change of ambient temperature. Short-term stability evaluation is usually carried out under different temperature conditions to investigate the effect of temperature on the undetermined characteristic values of standard substances. This experiment simulates the stability of the characteristic value of the matrix reference material under the condition of ice bag transportation. The short-term stability was investigated under the conditions of −37, 4, and 25 °C and storage in the dark for 0, 3, 7, 11, and 15 days. Three packages were taken each time, and two parallels were taken for each package. The average value of all data was calculated. The average value of the measurement results was used as the test results shown in Figure 5. The trend analysis was also used to analyze the monitoring data, and the results are shown in Tables S2−S4. The results showed that there was no trend change in the characteristic value of the matrix reference material within the monitoring time range, and the results fluctuated within the precision range of the measurement method. It was considered that the matrix reference material was stable at −37, 4, and 25 °C for 15 days in the dark, and the matrix reference material could be transported at room temperature. It should be noted that the fluctuation range of ciprofloxacin is slightly higher than that of the other two quinolones, which may be caused by the conversion of several other quinolones during storage.

4. CONCLUSIONS

In summary, we proposed an eco-friendly spiking method based on microfluidic technology, which is suitable for the development of matrix reference materials related to aquatic products. This method has three significant advantages: first, this method can significantly reduce drug loss and improve drug utilization. This method does not require subsequent drug residue treatment work, reducing the use of adsorbent materials or some organic reagents. Second, this method can significantly improve the drug-loading capacity of the matrix. Compared with traditional spiking methods, such as the medicated bath method and spraying method, this method can improve the adsorption efficiency of minced fish samples to drugs, thereby increasing the drug-loading capacity. Third, the matrix reference material prepared by this method shows good stability. Under the simulated real transportation conditions,

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the content of the drugs in the matrix reference material showed a stable trend, which met the real storage and transportation conditions.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c01874.

t-test results of the drug concentration, picture of minced fish gel microspheres, and standard curves of the drugs (PDF)

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Author Contributions

H.W.: investigation. H.L.: review and editing, conceptualization, supervision. H.S.: supervision. C.Z.: conceptualization, methodology, writing-review and editing.

Notes

The authors declare no competing financial interest.

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