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Determination of quinolones in wastewater by porous β-cyclodextrin polymer based solid-phase extraction coupled with HPLC

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ABSTRACT

In this research, a novel insoluble sorbent based on cyclodextrin and rigid aromatic groups tetrafluoroacetonitrile was designed for dispersive insoluble solid-phase extraction (DSPE). Due to its high adsorption capacity, this obtained polymer was applied to separation and concentration of trace quinolones in wastewater before HPLC determination. Various parameters influencing the extraction performance were studied and optimized. A DSPE approach coupled with high performance liquid chromatography was developed for the determination of four quinolones in wastewater samples. The limit of quantitation of fleroxacin, ciprofloxacin, gatifloxacin, norfloxacin were 2.67, 3.17, 4.75, 5.50 ng mL^{-1}, respectively. The recoveries of four quinolones range from 96.43 to 103.3% with relative standard deviations less than 4.5%. These results demonstrated that the proposed approach based on CDP was efficient, low-cost for extraction of quinolones from wastewater.

1. Introduction

In past several decades, antibiotics are widely used in prophylaxis and treatment of human and veterinary diseases [1]. The quinolones are an important class of synthetic antibiotics which have broad-spectrum activity against both gram-positive and gram-negative bacteria. They are broadly used to treat humans and food producing animals [2,3]. The main mechanism of quinolones is to affect two enzymes, DNA gyrase which relax the DNA in the replication fork and topoisomerase IV which It mediates the separation of offspring chromosomes. Consequently, bacterial multiplication is blocked [4,5]. The increase in the antibiotics resistance of pathogenic bacteria has become a serious problem, and the main reason is derived from heavy use and pollution in environment [6,7]. It has been issued relevant policies and regulations to control the abuse of antibiotics. Wastewater from the pharmaceutical factory, hospital and aquaculture cause serious pollution. Meanwhile, most of quinolones are excreted in urine or feces [8], which continue spread through soil and water [9,10]. Finally, these quinolones will damage human healthy through agriculture and water [11] (showed in the Scheme 1). During the process, the development of novel techniques for quinolones removal and determination in waste water become highly desired and very significant.

For trace substance detection, enrichment the analytes prior to analysis is necessary. Solid-phase extraction (SPE) has turned into the most commonly used sample preparation technique with the advantages of simple, rapid, solvent consumption and easy to combine with other technologies. Adsorbent plays a key role in the extraction process. Many materials, such as silica gel, fiber, chelating resin, activated carbon, and magnetic graphene oxide and carbon nanotube, were well researched [12–15]. Cyclodextrins are torus shaped cyclic oligosaccharides linked by several α-1, 4-anhydroglucopyranose units that simultaneously possess hydrophobic inner cavities and hydrophilic outer sides. The unique structures of CDs allow them to form host–guest inclusion complexes with suitably sized non-polar aliphatic and aromatic compounds through a series of forces such as hydrogen bonding, hydrophobic and van der Waals interaction [16]. A number of materials based on β-cyclodextrin were studied in solid-phase extraction field because of proper cavity and low price [17–19]. Natural β-cyclodextrin has certain solubility in aqueous solution, which limits its application in the field of adsorption. So, improvement of insolubility become the

Abbreviations: ACN, acetonitrile; CD, cyclodextrin; CDP, porous β-cyclodextrin polymer; DMF, *N*,*N*-dimethylformamide; DNA, deoxyribonucleic acid; EtOH, ethyl alcohol; FT-IR, fourier transform infrared spectroscopy; HAC, acetic acid; HPLC, high performance liquid chromatography; MeOH, methyl alcohol; SPE, solid-phase extraction; SS-NMR, solid-state NMR; THF, tetrahydrofuran

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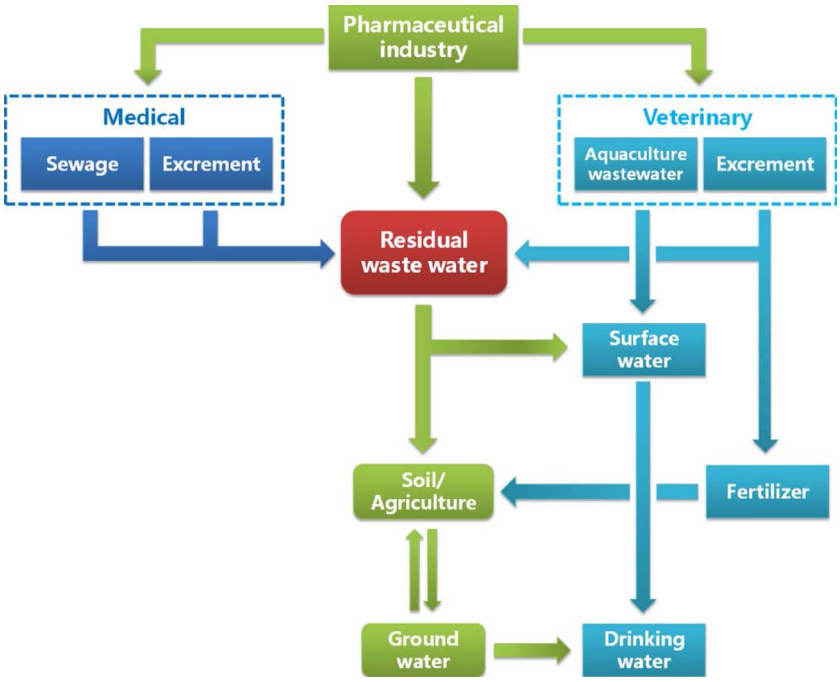

Scheme 1. Schematic diagram of quinolones pollution.

crucial point for cyclodextrin in SPE filed. At present, there are main aspect in the development of cyclodextrin materials. One is to immobilize the cyclodextrin onto other materials to form a complex, such as titanium dioxide [20], Fe_3O_4 [21], graphene oxide [22], gold [22], quantum dots [23], silicon dioxide [24]. This type materials always suffer from low proportion of cyclodextrin in complex and low yield high cost and difficulty in synthesis. Another method is to form a polymer with a cyclodextrin as a unit through a crosslinking agent, which include epichlorohydrin [25], pyromellitic dianhydride [26], citric acid [27], chitosan [28], polyethylene glycol [29], carboxymethylcellulose [30], *N,N′*-methylenebisacrylamide [31] and hexamethylene diisocyanate [32]. These polymers tend to dissolution due to their soluble crosslinking agent. So, they are usually used for drug carriers rather than solid phase extractants. Recently, a new β-cyclodextrin polymer which is cross-linked by rigid aromatic group tetrafluoroterephthalonitrile form permanent porosity was reported. The polymer not only possesses a high-surface-area, mesoporous structure, but also has unique properties insolubility and good dispersibility, which makes it superior to activated carbons [33]. It can rapidly sequester a variety of aromatic compounds micropollutants in water, adsorption rate constants for bisphenol A is 15–200 times greater than activated carbons [33]. More than that, another remarkable focus is cheap, re-utilizing and non-polluting. All of these advantages bring it commercial application value for removing contaminants. If the polymer can be used as a solid phase extraction material, it is potential to build a method of trace substance in environment, medicine and food filed. So far, there has been no systematic study of this aspect.

In the work described here, to establish an efficient analytical method, a porous cyclodextrin polymer(CDP) has been used as SPE sorbents for the extraction of four quinolones from waste water samples. All parameters that could affect the extraction efficiency were optimized. The results revealed that this method is feasible for quinolones detection in wastewater.

2. Experimental

2.1. Chemicals and materials

B-Cyclodextrin (> 98%), Tetrafluoroterephthalonitrile (> 99%) were purchased from Aladdin Reagent Co., Ltd., China. K_2CO_3, 1,4-epoxy-butan, *N,N*-Dimethylformamide, Methanol in analytical purity were purchased from Sinopharm Chemical Reagent Co. Ltd. Four quinolones (fleroxacin, norfloxacin, ciprofloxacin, gatifloxacin) were supplied by National Institutes for Food and Drug Control.

2.2. Instruments

FT-IR spectrum was collected by using 8400 s FTIR spectrometer in KBr pellet at room temperature (Shimadzu Corporation, Japan). The HPLC analyses were performed on a Shimadzu LC-20AT HPLC system including a binary pump and a diode array detector (Shimadzu, Kyoto, Japan). The analytical column was a Hyperisl ODS2 C18 (250 mm × 4.6 mm, 5 μm). The pH measurements were performed with a pH S-25 digital pH-meter (Shanghai Wei Ye Instrument Factory, China) with a combined glass electrode.

2.3. Preparation of CDP

CDP was synthesized according to the published method with little modification [33]. The procedure was as follows: a dried 100 mL serum bottle was flushed with N_2 gas for 5 min, then was charged with β-CD (1.025 g), Tetrafluoroterephthalonitrile (0.5 g), and K_2CO_3 (1.6 g). An anhydrous THF/DMF mixture (9:1 v/v, 40 mL) was added, the vial was sparged with N_2 for additional 5 min, then placed in the magnetic stirring thermostatic oil bath (85 °C, 500 rpm for 2 d). The suspension was filtered, K_2CO_3 was neutralized with 1 M HCl. After centrifugation, the light-yellow solid was recovered and activated by soaking in H_2O (2 × 10 mL) for 15 min, THF (2 × 10 mL) for 30 min and CH_2Cl_2 (1 × 15 mL) for 15 min. The final product was freeze-dried.

2.4. Choice of initial pH

2 mg of CDP were added into 1 mL of different quinolones solution with concentration of 200 μg mL^{-1}, respectively. The pH of the solution was adjusted to 2, 4, 6, 8 and 10. The concentration of free quinolones in the supernatant was measured by UV–vis spectrometer at 285 nm with spectral scanning from 250 to 400 nm.

2.5. The adsorption kinetics of quinolones

The adsorption kinetics of the different quinolones toward CDP was investigated by varying adsorption time from 0 to 120 min. 2 mg of CDP were mixed with 2 mL of quinolones at a concentration of 200 μg mL^{-1}. The concentration of quinolones in the supernatant was measured by UV–vis spectrometer at 285 nm with spectral scanning from 250 to 400 nm. The amounts of the quinolones bound to CDP were calculated by subtracting the quinolones concentrations from the initial solution added before binding preparation to the supernatant.

Pseudo-first-order kinetics model and Pseudo-second-order kinetics model were applied to describe the adsorption.

The linear form of pseudo-first-order kinetic equation [34] is

$$\ln(q_e - q_t) = \ln q_e - k_1 t$$

The linear form of pseudo-second-order kinetic equation [35] is

$$\frac{t}{q_t} = \frac{t}{q_e} + \frac{1}{k_2 q_e^2}$$

where q_t and q_e are the adsorbate uptakes (mg adsorbate per g polymer) at time q_t (min) and at equilibrium, respectively, and k_2 is an apparent second-order rate constant (g mg^{-1} min^{-1}).

2.6. Adsorption isotherm

The adsorption isotherm was performed by suspending 2 mg of CDP in 3 mL of the quinolones solutions at pH 4 with different concentrations ranging from 10 to 1000 μg mL^{-1}. After ultrasound for 5 min, the mixtures were kept for 1 h at room temperature with shaking. Then the mixture was separated by centrifugation. The concentration of free quinolones in the supernatant was measured by UV–vis spectrometer as previously mentioned.

According to the variance of the quinolones concentrations before and after adsorption, the equilibrium adsorption capacity (Q, μg mg^{-1}) of the quinolones bound to CDP is calculated by

$$Q = (C_0 - C_1)\cdot V/m$$

where C_0, C_1, V, and m represent the initial solution concentration of the analyte, the final solution concentration (μg mL^{-1}), the volume of the solution (mL), and the weight of the polymer (mg), respectively. The average data of triplicate independent results were used for the following discussion.

2.7. Sample preparation

Standard stock solutions of fleroxacin, norfloxacin, ciprofloxacin, and gatifloxacin at a concentration of 1000 μg mL^{-1}, were prepared in methanol and then diluted to the desired concentration. Appropriate milliliters of stock solutions of fleroxacin, norfloxacin, ciprofloxacin, and gatifloxacin were spiked to the simulated sample. The concentrations of simulated samples were prepared with 2.5, 0.5 and 0.1 μg mL^{-1} of each quinolone drug, respectively. Sewage was collected from the Hospital of China Pharmaceutical University and filtered with ultrafiltrate membrane.

2.8. SPE based on CDP

The SPE procedure using CDP allowed rapid pre-concentration of analytes in wastewater samples by centrifugation method. Firstly, 8 mg of CDP were added into a centrifuge tube with 20 mL of simulated samples. The mixture was vortexed for 1 min in an oscillator for uniform dispersion. Subsequently it was shaken for 1 h to reach desorption equilibrium. The tubes were centrifuged at 13,000 rpm for 10 min. After removing the supernatant, the residual was dried with N_2. Then 0.5 mL desorption reagent ACN-1%HAc (6:4) were added to centrifuge tube. After 20 min ultrasonication, the determination and were washed away, then separated by centrifugation. The suspension was dried by N_2. After redissolve with MeOH, solution was filtered by a Whatman 0.2 μm inorganic membrane filter. The filtrate was then measured by HPLC. The experiments were conducted in duplicate/triplicate.

2.9. HPLC-UV analysis of samples

The mixed solution containing norfloxacin, ciprofloxacin, gatifloxacin solution at the concentration of 200 μg mL^{-1} was diluted to different working standard solutions in a range of (0.1–200 μg mL^{-1}). The peak area of each compound was measured by high performance liquid chromatography. The four standard curves were constructed by plotting the peak area against the nominal concentration of each compound. Then, the concentration of the sample solution was calculated by detecting the peak area of the sample solution. The mobile phase consisted of methanol-0.025 M aqueous phosphoric acid solution (20:80, V/V, pH = 3.2) and the flow-rate was set at 1 mL min^{-1}. Column temperature was 40 °C. The injection volume was 20 μL, and the effluent was analyzed by HPLC equipped with DAD detector at 285 nm.

2.10. Optimization of extraction conditions

Variable factors affected the adsorption and desorption procedure were investigated. The adsorption and desorption process was optimized through adjusting some parameters like: amount of adsorbent (interval from 4 to 20 mg), the eluent composition (acetone, methanol, ethanol, acetontrile-1% acetic acid) and desorption mount (interval from 0.5 to 2.5 mL) were optimized. When one parameter was changed, the other parameters were kept at their optimal values.

3. Results and discussion

3.1. Characterization

SS-NMR (400 MHz) chemical shifts δ of CDP were shown in Fig. 1A. The signal at 73.26 ppm and 103.9 ppm respectively represent C3 and C1 in CD. The signal at 163.9 is correlated with carbon atom connect with F in tetrafluoroterephthalonitrile. The resonances at 97.42 ppm correspond to the newly formed alkoxy groups and 142.67 ppm correspond to aromatic carbons. This is successfully synthesized, which was in good agreement with the reported description [33,36].

The infrared spectra of CDP are shown in Fig. 1B, indicating that CDP was successfully synthesized. The characteristic peaks −C≡N of linker were 2250 cm^{-1}.There was a weak peak at 1268 cm^{-1}, which present C−F stretches, which illustrated that F was not completely substituted. The adsorption band at 1463 cm^{-1} were attributed to C−C aromatic stretches. The O−H stretches were near 3330 cm^{-1}, aliphatic C−H stretches around 2933 cm^{-1}, and an intense C−O stretch at 1033 cm^{-1}.

3.2. Choice of initial pH

The pH value of sample solution plays an important role in extraction process by determining the existing state of analytes as ionic or molecular state as well as the surface properties of absorbent material. In this study, effect of pH value on the recoveries in the range of 2–10 was investigated, firstly. As showed in Fig. 2A, the recoveries of all quinolones at pH 4 were the highest, and at pH 10 were the lowest. Such a result is easy to explain. The guest/host binding of cyclodextrin and the quinolones is mainly due to hydrophobic interaction [37–39]. The quinolone antibiotics are amphoteric compounds which possess two pKa [40]. When the quinolones as molecular state is compatible with inclusion in the nonpolar cyclodextrin cavity. In acidic pH condition, the case that the quinolones molecules are in the cationic form

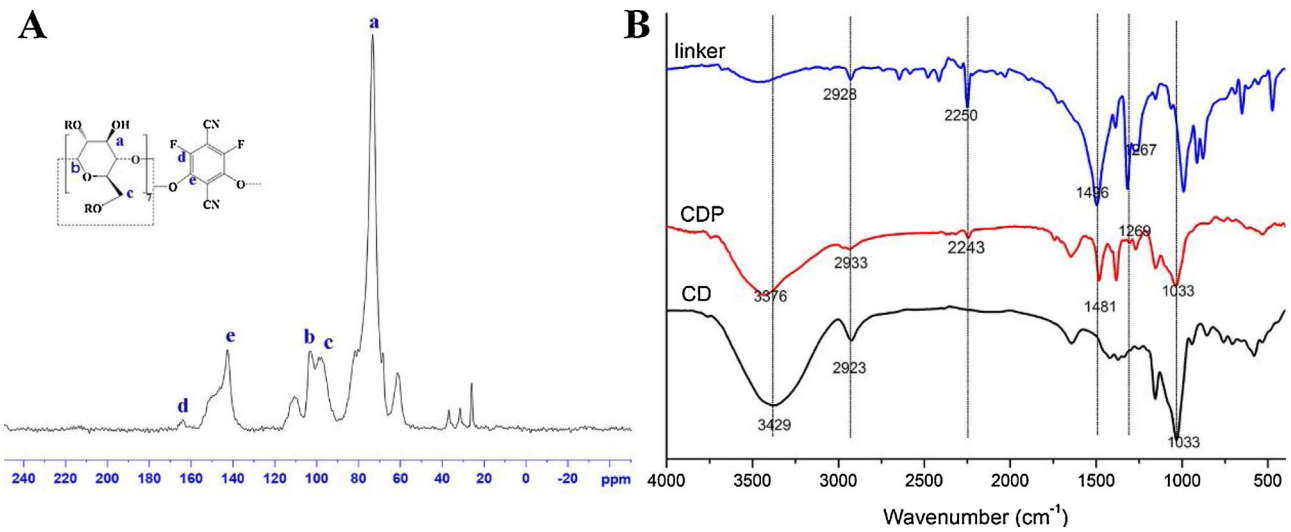

Fig. 1. A.^{13}C CP-MAS solid-state NMR spectra of CDP. B. Infrared spectra of CDP and CD and linker.

and the protonation of hydroxide of cyclodextrin reduce interaction between of cyclodextrin and the quinolones. In alkaline condition, quinolones have negative charge, the hydroxide of cyclodextrin occurs ionization form alkoxy ion with negative charge as well [41], electrostatic repulsion lower absorption.

3.3. Kinetic adsorption curve of CDP

The kinetic adsorption curve of CDP about different quinolones is shown in Fig. 2B. During the first 20 min, adsorption rate increased rapidly and equilibrium adsorption was reached after 50 min.

When pseudo-first-order equation was used to describe adsorption, the equilibrium adsorption capacity (q_e) of fleroxacin, ciprofloxacin, gatifloxacin, norfloxacin were 27.56, 48.50, 47.38 and 30.09 μg mg^{-1},

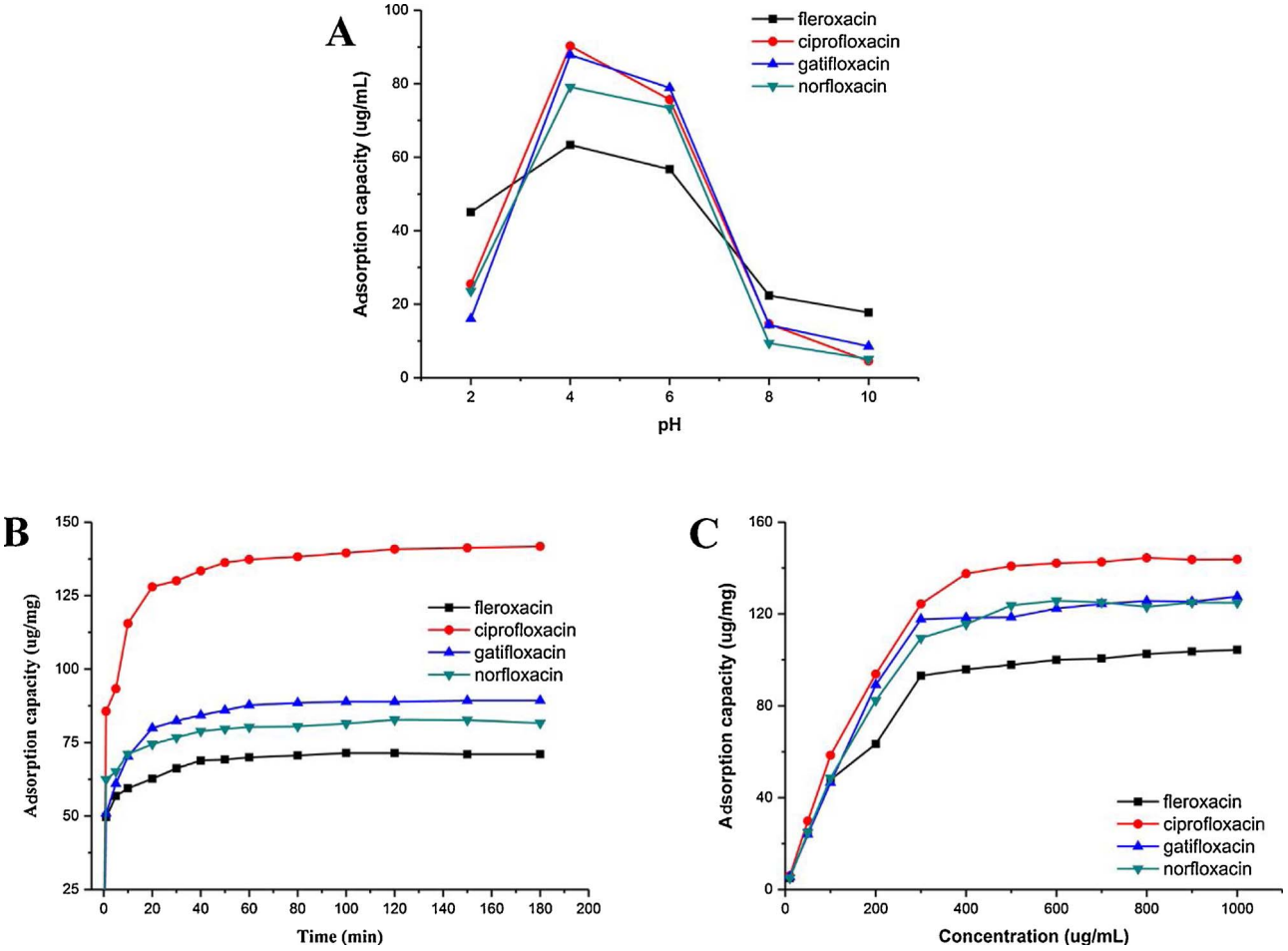

Fig. 2. A. The effect of pH for absorption capacity. B. Adsorption dynamic curves of different quinolones. C. Adsorption isotherm curves of different quinolones.

Table 1
Adsorption kinetic constants of models.

		Pseudo-first-order kinetic model			Pseudo-second-order kinetic model		
	Q	qe (μg mg^{-1})	K1	R^2	q_e (mg g^{-1})	K_2 (g mg^{-1} min^{-1})	R^2
fleroxacin	69.94	27.56	0.0716	0.9865	65.79	0.0051	0.9988
ciprofloxacin	137.29	48.50	0.0740	0.9614	101.01	0.0023	0.9974
gatifloxacin	87.75	47.38	0.0708	0.9713	96.15	0.0023	0.9986
norfloxacin	80.30	30.09	0.0794	0.9849	83.33	0.0052	0.9998

respectively, much lower than the actual amount Q of equilibrium adsorption (69.94, 137.29, 87.75 and 80.30 μg mg^{-1}), however, the results from Pseudo-second-order kinetic model is approximate. Pseudo-second-order kinetic model display R^2 was 0.9988, 0.9974, 0.9986 and 0.9998, which indicated the high correlation (Table 1). It was found that the second-order equation model provided higher correlation coefficients with experimental results. The absorption is deemed to be effected to chemisorption.

3.4. Adsorption isotherm of CDP

The adsorption isotherms of CDP with different quinolones are shown in Fig. 2C. Obviously, the adsorption of CDP all increased with the increase of the concentration of quinolones varying from 0 to 400 μg mL^{-1}.

Langmuir and Freundlich are models generally used to study adsorption isotherms. The Langmuir isotherm describes that surface as homogeneous assuming that all the adsorption sites have equal solute affinity and that adsorption at one site does not affect the adsorption at an adjacent site. The equation is as follows:

$$\frac{C_e}{Q} = \frac{Ce}{Q_m} + \frac{1}{Q_m K_L}$$

where Q is the equilibrium adsorption amount (μg mg^{-1}), Ce is the residual concentration at equilibrium (mg mL^{-1}), Qm is the maximum adsorption capacity predicted (μg mg^{-1}), K_L is Langmuir constant (mL μg^{-1}), which is related to the affinity of binding site.

The Freundlich isotherm assumes that the ion adsorption occurs on a heterogeneous surface by multilayer adsorption. The isotherm equation is as follows:

$$\lg Q = \lg K_F + \frac{lgC_e}{n}$$

where Q is the equilibrium adsorption amount (μg mg^{-1}), Ce is the residual on centration at equilibrium (mg mL^{-1}), K_F and n are Freundlich constants representing adsorption capacity and strength respectively.

According to Langmuir and Freundlich models, the equilibrium adsorption of CDP about different quinolones was investigated. The correlation coefficients and constants were calculated and showed in Table 2. The linear correlation coefficients (R^2) in the Langmuir were 0.9918, 0.9969, 0.9959 and 0.9953 for fleroxacin, ciprofloxacin, gatifloxacin and norfloxacin, respectively. The maximum adsorption amount were 101.01, 144.93, 126.58, 126.58 μg mg^{-1}. It is agreed to real value 95.88, 124.36, 118.35, 110.56 μg mg^{-1}. In the Freundlich isotherm model, the linear correlation coefficients were 0.8982, 0.9100, 0.9491 and 0.9172 lower than obtained R^2 for Langmuir. Considering this result, it can be seen that all of adsorptions towards quinolones were corresponding to Langmuir model which is suitable for homogeneous system.

Table 2
Freundilich and Langmuir isotherm model for quinolones adsorption on CDP at room temperature.

	Langmuir			Freundlich		
	Q_M	K_L	R2	1/n	K_F	R^2
fleroxacin	101.01	0.9016	0.9918	0.2952	20.34	0.8982
ciprofloxacin	144.93	0.1022	0.9969	0.3131	26.48	0.9100
gatifloxacin	126.58	0.0587	0.9959	0.3080	21.47	0.9491
norfloxacin	126.58	0.1145	0.9953	0.3274	23.00	0.9172

3.5. Optimization of SPE conditions

To evaluate the properties of CDP as SPE sorbent for extraction of the quinolones, variable parameters affecting the extraction efficiency were investigated including the amount of CDP, eluent and adsorption mount, desorption time. When one parameter was changed, the other parameters were kept at their optimal values.

3.6. Effect of the mount of adsorbent

During the extraction process, the amount of CDP is one of the important factors. The adsorbent specific surface area and availability of more adsorption sites increase with the increase in adsorbent amount. With the increase of adsorbent amount to a degree, if the total of concentration of adsorbate keep constant in this system, the further increase of adsorbent amount will reduce the amount of adsorption per unit amount. Different amounts ranging from 4 to 20 mg was studied. As show in Fig. 3A, when adsorbent amount at 4 mg, different quinolones has different recovery, and all are more than 86%. At the point of 8 mg, all the quinolones recovery exceeded 94%. So, given the findings, 8 mg of CDP was used in the next experiments.

3.7. Effect of desorption conditions

The desorption of quinolones from CDP was studied to use different organic solvents such as acetone, methanol (MeOH), ethanol (EtOH) and acetontrile-1% acetic acid (6:4) (CAN-HAC). From Fig. 3B, it could be found that the desorption ability of acetonitrile containing 1% acetic acid is superior to other solvents.

Fig. 3C showed that 1.5 mL was applicable mount for achieving the more efficient desorption condition.

The effect of desorption time from 5 to 25 min on the recoveries of the target analytes was investigated. As shown in Fig. 3D when desorption time was 5 min, the desorption recoveries were more than 50%. In the rest period of time, it can be obviously found that the extraction recoveries mushroom. After 20 min, it reached the equilibrium.

3.8. Analytical performance and application

To evaluate the accuracy and feasibility of the method developed, quantitative parameters of the method, such as linear range, correlation coefficient, limit of detection (LOD), limit of quantitation (LOQ) and recovery were evaluated.

3.8.1. Linearity, LOD and LOQ

The linearities of the analytes were studied in wake water samples. All the parallel experiments were repeated three times. Limits of detection (LODs) of the investigated compounds were estimated as the

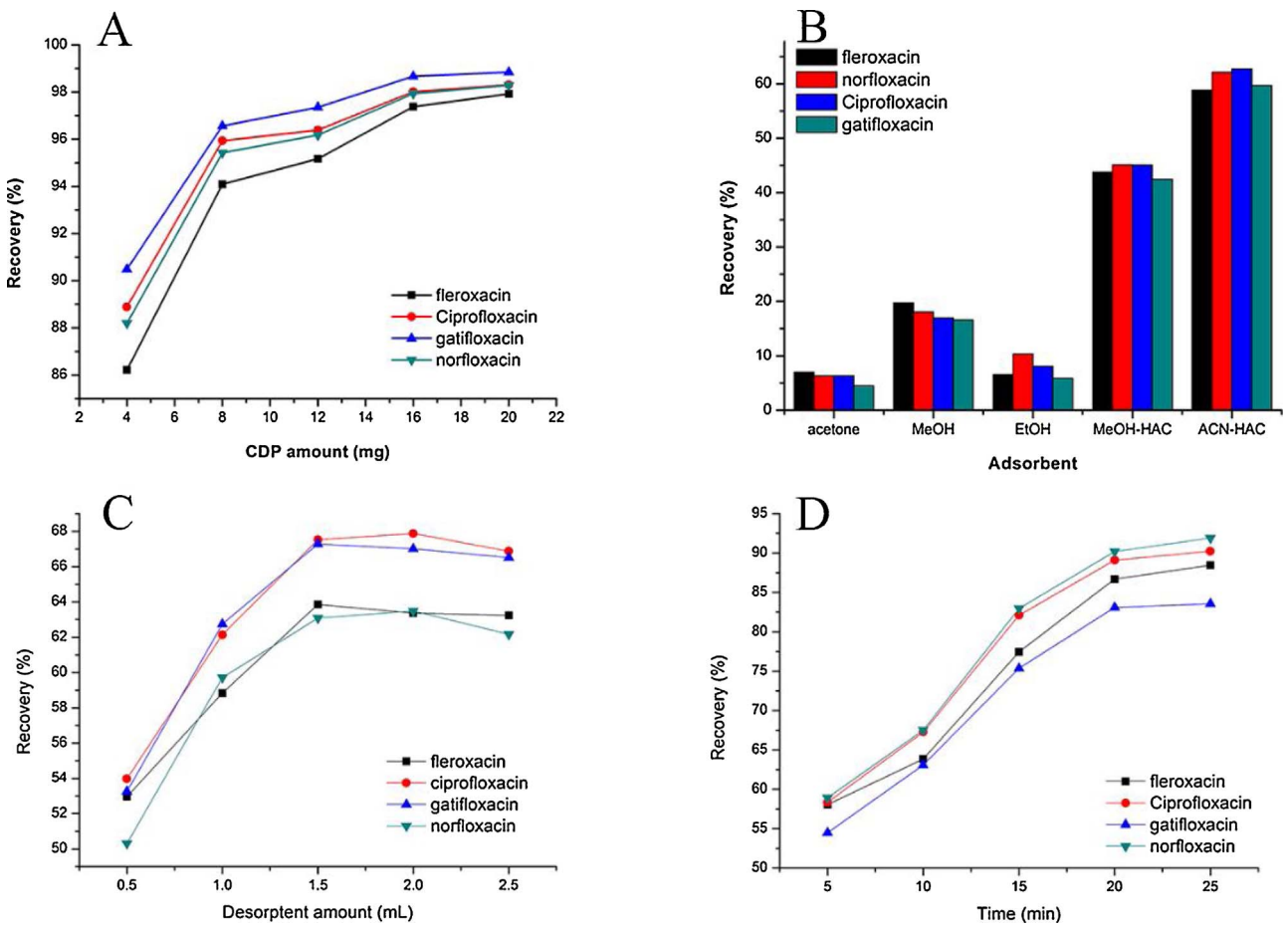

Fig. 3. A. The effect of CDP amount. B. the effect of absorbent. C. The effect of desorption time. D. The effect of eluent.

Table 3
Analytical parameter results of four quinolones.

	Liner range(ng/mL)	R^2	LOQ (ng/mL)	LOD (ng/mL)
fleroxacin	25–5000	0.999	8.95	2.67
ciprofloxacin	25–5000	0.999	10.5	3.17
gatifloxacin	25–5000	0.997	15.7	4.75
norfloxacin	25–5000	0.994	18.9	5.50

minimum concentration determined with a signal-to-noise ratio of 3 and the LOQ values taken by signal-to-noise ratio of 10. The linear range, correlation coefficients (R^2) and LODs are listed in Table 3. The results showed the linear ranges of 25–5000 ng mL^{-1}. All of the analytes exhibited good linearity with satisfactory regression coefficients (R^2 = 0.9994). The limits of detection (LOD) for fleroxacin, ciprofloxacin, gatifloxacin, norfloxacin were 2.67, 3.17, 4.75 and 5.50 ng mL^{-1} respectively.

3.8.2. Precision and accuracy

The recovery of the method was validated using the standard addition method. Table 4 gave the recoveries calculated after spiking three different concentration levels (low, middle and high concentrations) of three target analytes. The recoveries were expressed as the mean value of three independent determinations. Recoveries were in the intervals 95.47–99.20%, 96.09–101.86%, 98.01–101.89% and 96.43–103.3% forfleroxacin, ciprofloxacin, gatifloxacin and norfloxacin, respectively, with the relative standard deviations of the recoveries varying between 0.81% and 4.44%. These results indicated that the present method has good accuracy and precision.

3.8.3. Robustness

Robustness of the method was determined a standard solution at 2500 ng mL^{-1} by changing some analytical conditions such as mobile phase ratio, flow rate, oven temperature, and pH of mobile phase [42]. Here, considering that there were extraction conditions optimization experiments, robustness study was only for chromatographic conditions. Results are expressed in Table 5, negligible differences in these

Table 4
Precision and accuracy for detection of analytes in wastewater sample.

	intra-day			inter-day		
	2.5	0.5	0.1	2.5	0.5	0.1
fleroxacin	99.20 ± 1.03	96.86 ± 2.85	96.87 ± 4.44	98.65 ± 2.46	95.47 ± 3.07	95.77 ± 3.45
Ciprofloxacin	101.86 ± 1.88	96.09 ± 3.20	97.22 ± 4.44	97.56 ± 2.96	97.45 ± 2.36	97.32 ± 3.45
gatifloxacin	99.10 ± 1.85	100.03 ± 3.77	97.97 ± 3.35	98.67 ± 3.26	101.89 ± 2.56	98.01 ± 3.51
norfloxacin	96.43 ± 0.81	101.95 ± 1.93	99.43 ± 3.35	100.13 ± 3.56	103.3 ± 3.45	101.35 ± 2.17

Table 5
Robustness study.

Chromatographic changes	Level	fleroxacin			norfloxacin			ciprofloxacin			gatifloxacin		
		Area	N	TF	Area		FT	area		TF	Area		TF
Mobile phase ratio	19:81	212,459	52,478	1.06	259,509	59,534	1.08	228,051	59,274	1.02	142,623	59,813	1.09
	20:80	192,144	52,140	1.05	253,745	56,782	1.09	218,037	55,074	1.0	132,494	51,848	1.10
	21:79	205,179	51,366	1.05	248,442	56,957	1.09	232,761	55,880	1.01	133,881	60,794	1.08
Mean ± SD		203,260 ± 8403			253,898 ± 4519			226,283 ± 6139			136,332 ± 4483		
RSD (%)		4.13			1.78			2.71			3.28		
pH of mobile phase	3.1	194,821	46,428	1.02	241,746	49,855	1.07	230,113	47,686	1.02	133,414	43,502	1.13
	3.2	192,144	52,140	1.05	253,745	56,782	1.09	218,037	55,074	1.00	132,494	51,848	1.10
	3.3	186,895	47,893	1.06	251,249	50,134	1.10	212,526	51,237	1.02	127,401	56,694	1.09
Mean ± SD		191,286 ± 3292			248,913 ± 5169			220,225 ± 7344			131,103 ± 2644		
RSD (%)		1.72			2.07			3.35			2.01		
Flow rate (mL/min)	0.9	202,733	54,068	1.10	261,542	59778	1.11	235,086	58,159	1.02	149,451	58,890	1.12
	1	192,144	52,140	1.05	253,745	56782	1.09	218,037	55,074	1.00	132,494	51,848	1.10
	1.1	183,253	52,374	1.02	239,645	56756	1.05	209,582	56,915	1.00	128,162	60,108	1.09
Mean ± SD		192,710 ± 7962			251,644 ± 9062			220,901 ± 10,607			136,702 ± 9186		
RSD (%)		4.13			3.6			4.08			6.7		
Column temperature (°C)	35	208,408	49,563	1.08	255,330	55,254	1.10	244,387	53,946	1.02	137,312	56,276	1.11
	40	192,144	52,140	1.05	253,745	56,782	1.09	218,037	55,074	1.00	132,494	51,848	1.10
	45	213,705	56,806	1.06	280,164	61,651	1.08	247,689	60,570	1.01	150,542	60,394	1.06
Mean ± SD		204,752 ± 9173			263,079 ± 12,097			236,704 ± 13,268			140,116 ± 7630		
RSD (%)		4.48			4.59			5.6			5.4		

Area: peak area; N: theoretical plates/mete; FT: tailing factor.

parameters were observed, the RSD% of peak area were less than 6.7%. The results provide an indication of its reliability during normal usage. And it also demonstrated the very good performance of the analytical method.

3.8.4. Analysis of samples

In order to validate the suitability of the developed method, the method was applied to analyze the analytes fleroxacin, ciprofloxacin, gatifloxacin and norfloxacin in waste water samples. The recovery of the method was validated by the standard addition method. The reference standards were added at three different concentration levels (low, middle and high concentrations of the matrix) with five parallel experiments at each level. All the parallel experiments were repeated three times and the results are tabulated in Table 6. It could be seen that recoveries ranging from 96.43% to 101.95% for the four target analytes was obtained. Thus, the proposed method is suitable for analyzing the wastewater samples. Chromatogram is shown in Fig. 4. It can be seen that no interferences in waste mater sample. The results implied the proposed method based on CDP was excellent specificity for the determination of fleroxacin, ciprofloxacin, gatifloxacin and norfloxacin.

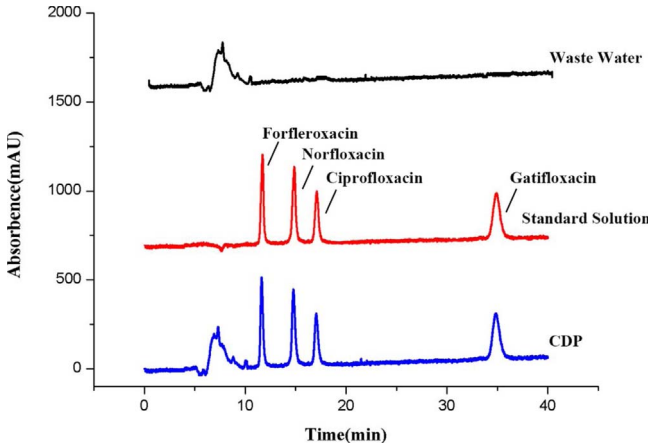

Fig. 4. HPLC–DAD chromatograms of samples: the black, red and blue lines represent the waste water, standard solution, solution recovered by CDP. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.9. Comparison of different methods applied to extract the quinolones

As the concentration of quinolones (ng L^{-1}) in the environmental waters is usually very low, HPLC-UV whose detection limit is micrograms per milliliter level is difficulty to detection [43,44]. Hence, quinolones in environmental water samples are mainly carried out by HPLC coupled to mass spectrometry or tandem mass spectrometry or fluorescence detection [45]. Despite of the combination of highly sensitive detectors, SPE is still an essential step. Using commercialized cartridges, like columnOasis® HLB cartridge and Bondesil C18 cartridge, to enrichment and purification is the most popular, but there are shortcomings, including cost-expensive, time-consumer, low recovery, interference, strong acidic pH condition [44–49]. Of course, there are new methods without need of extraction to be reported, such as electromembrane extraction [49]. However, they also encountered cost-expensive operation complicated. In comparison with other methods (Table 7) [43–52], the present method shows advantages of higher extraction efficiency, low cost, comparable detection limit, good recoveries and relative wider linear range.

Table 6
Recoveries obtained for the determination of the analytes wastewater sample.

Analytes	Spiked (ug/mL)	Mean accuracy (%)	RSD%
fleroxacin	0.1	96.87	4.16
	0.5	96.86	2.85
	2.5	99.20	1.03
Ciprofloxacin	0.1	97.22	4.44
	0.5	96.09	3.20
	2.5	101.86	1.88
gatifloxacin	0.1	97.97	3.35
	0.5	100.03	3.77
	2.5	99.10	1.85
norfloxacin	0.1	99.43	3.50
	0.5	101.95	1.93
	2.5	96.43	0.81

Table 7
Comparison of different methods applied to extract the quinolones.

Sample	Extraction method	Determination method	LR	LODs	RSDs (%)	Recovery (%)	Ref.
tablet		HPLC-UV	4.0–24.0 μg mL^{-1}	0.13 μg mL^{-1}	0.17	105.89	43
pig plasma	SPE	CZE	5–20 μg mL^{-1}	1.1, 2.4 μg mL^{-1}	4.1–4.2	94.06,123.36	44
human urine	SPE	HPLC-DAD	39–1260 ng mL^{-1}	35.59 ng mL^{-1}	4.87, 7.99	42.47,41.82	45
waste water	SPE	UPLC–MS/MS	0.02–0.04 ng mL^{-1}	0.07–0.15 ng mL^{-1}	1.0–2.8	99.1–102.6	46
waste water	SPE	UHPLC-FD	150.00–5000.00 ng mL^{-1}	50.00 ng mL^{-1}	0.22–4.99	80.63–128.95	48
waste water	SPE	UHPLC–MS/MS	1.00–500.00 ng mL^{-1}	0.50 ng mL^{-1}	5.0–18.71	98–100	49
environmental water	SPE	HPLC-FD	0.02–0.30 ng mL^{-1}	0.001–0.080 μg mL^{-1}	7.1	82.1–125.8%	50
water sample	SPME	HPLC-FD	0.02–0.23 ng mL^{-1}	0.01–0.2 ng mL^{-1}	6.8	81–116%	51
wastewater	Electromembrane extraction	HPLC–DAD	70–10^6 ng L^{-1}	40–70 ng L^{-1}	1.7–2.6	73.6–93.1	52
waste water	SPE	HPLC–DAD	25–5000 ng mL^{-1}	3.17 ng mL^{-1}	1.88–4.4	96.09–101.86	present

4. Conclusion

In this paper, a method with porous cyclodextrin polymers was established as extractant to detect four kinds of quinolones. Through evaluated in a series of adsorption experiments, higher extraction efficiency, comparable detection limit, good recoveries and relative wider linear range were obtained. This method employed solid phase extraction and HPLC-DAD was successfully applied to detect quinolones in wastewater in case of low concentration. In addition, another significative trait of this mothed should be pointed out that the reagents and materials used in this study were low-cost and environmentally friendly. Although the method using CDP as absorbent has the proposed advantages, centrifugal operation of the whole process is troublesome. If magnetism can be introduced into the material, it will greatly simplify the operation. The results of this article and the flow-through experiment in *Rapid removal of organic micropollutants from water by a porous β-cyclodextrin polymer* indicate that it may be accomplished by filling the CDP into a small column and may be used for analysis of larger volume samples. These will be further studied in the next work. The results reveal that the material has a great application potential for using as a simple and efficient extraction and preconcentration technique for trace substances in wastwater samples in environment.

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