Supporting Information

Nitrogen-doped graphene quantum dots coated with molecularly imprinted polymers as a fluorescence sensor for selective determination of warfarin

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Experimental section

Materials and reagents

Duloxetine hydrochloride, 3-Aminopropyltriethoxysilane (APTES), thalidomide, sertraline hydrochloride all were supplied by Aladdin Chemistry Co., Ltd. (Shanghai, China, https://www.aladdin-e.com). Tetraethyl orthosilicate (TEOS), ellagic acid, promethazine hydrochloride all were supplied by Macklin Co., Ltd. (Shanghai, China, http://www.macklin.cn). Quinine sulfate, coumarin were obtained from Energy Chemical (Shanghai, China, https://www.energy-chemical.com). Potassium chloride (KCl), sodium carbonate anhydrous (Na_2CO_3) were obtained from Shanghai Lingfeng Chemical Reagent Co., Ltd. (Shanghai, China). Citric acid monohydrate, ammonia solution (25%-28%) were obtained from Nanjing Chemical Reagent Co., Ltd. (Nanjing, China, http://www.nj-reagent.com). Urea, sodium chloride (NaCl), calcium chloride anhydrous ($CaCl_2$), sodium sulfate anhydrous ($NaSO_4$) were purchased from Xilong Science Co., Ltd. (Guangdong, China, http://www.xlhg.com). Warfarin, florfenicol were purchased from J&K Scientific Co., Ltd. (Beijing, China, https://www.jkchemical.com). Nifedipine was purchased from Meilun Biology Co., Ltd. (Dalian, China, http://www.meilune.com). Sodium hydrogen carbonate ($NaHCO_3$) was supplied by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China, https://www.sinoreagent.com). Sulfadimidine was supplied by the China National Institute of Pharmaceutical and Biological Products (Beijing, China). Magnesium chloride ($MgCl_2$) was obtained from Mairuier Chemical Technology Co., Ltd. (Shanghai, China, https://www.meryer.com).

Synthesis of NGQDs

Briefly speaking, 0.5 g of citric acid and 0.09 g of urea were dissolved in pure water under ultrasound. The mixtures were transferred into a Teflon-equipped stainless steel autoclave and then subjected to hydrothermal treatment at 200 ℃ for 8 h.[2] After cooling to ambient temperature, NaOH was added to adjust the pH of the solution at 7. The obtained yellowish solution was filtered by a 0.45 μm filter membrane to remove the large particle. Then the resulting solution was dialyzed using a dialysis bag with a molecular weight cutoff of 500 Da. Eventually, the NGQDs solution was preserved at 4 ℃ in refrigerator for further use.

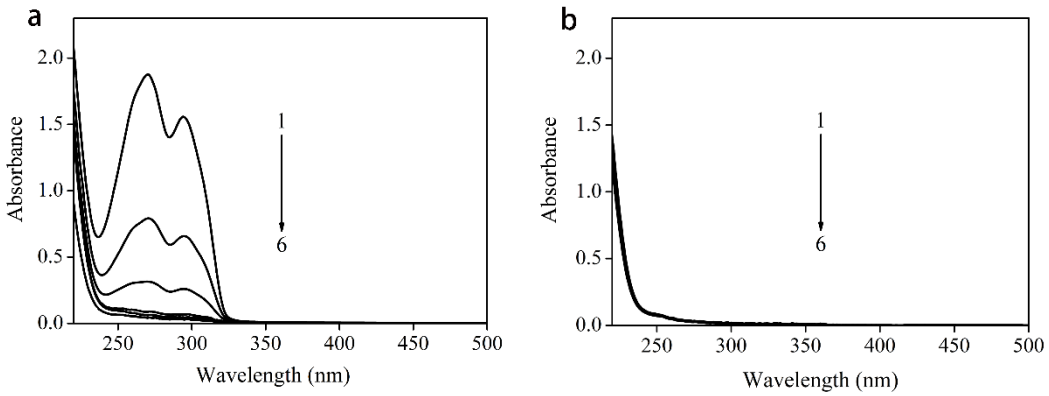

Fig. S1 UV-Vis spectra of extraction mixtures of MIPs (a) and NIPs (b) after each wash solutions.

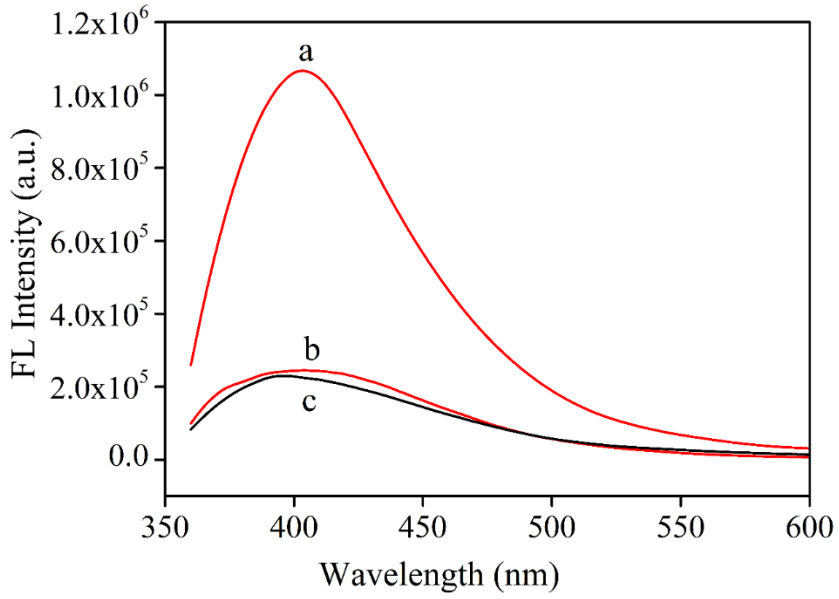

Fig. S2 Fluorescence spectra of NGQDs@MIPs before (a) and after (b) removal of templates and NGQDs@NIPs (c).

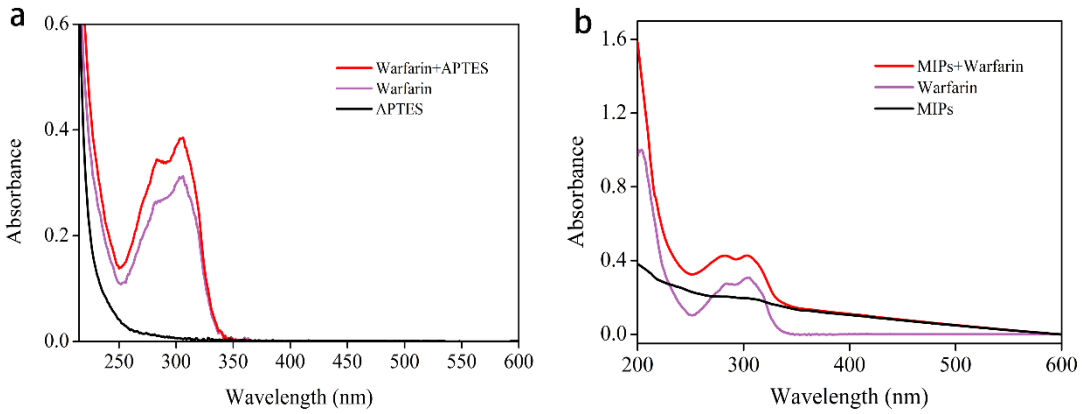

Fig. S3 (a) UV-Vis absorption spectra of the warfarin, APTES, and the mixture of them in EtOH; (b) The UV-Vis absorption spectra of NGQDs@MIPs, warfarin, the mixture of NGQDs@MIPs and warfarin.

Optimization of experimental parameters

Effect of amount of NGQDs@MIPs

The concentration of NGQDs@MIPs is a crucial factor that can affect the linear range and sensitivity of the warfarin detection system. Therefore, 0.025-0.5 mg mL^{-1} quantities of NGQDs@MIPs were used to examine the effect on fluorescence enhancement (Fig. S4a). When the concentration of NGQDs@MIPs is 0.1 mg mL^{-1}, the best sensor response can be identified. A small quantity of NGQDs@MIPs would result in a narrow linear range in the detection of warfarin, whereas the sensitivity of the sensor would decrease when the amount of NGQDs@MIPs is excessive.[3] Consequently, 0.1 mg mL^{-1} was adopted as the optimum NGQDs@MIPs quantity.

Effect of pH

Acidic or alkaline media can influence the binding potency of NGQDs@MIPs.[7] Then the pH effect on the response of the NGQDs@MIPs sensor to warfarin was evaluated

in the range of 2-12. As illustrated in Fig. S4b, the fluorescence intensity of the NGQDs@MIPs sensor gradually increases with the fluctuation of the pH value in the range of 2 to 6 in the presence of warfarin solution (2.5 μM), while the fluorescence intensity was decreased when the pH value exceeds 6. Therefore, the highest sensitivity of the sensor is at pH 6.0. The hydrogen ion will exert an influence on the hydrogen bonding between warfarin and NGQDs@MIPs sensor when they are in the strong acidic media. And the hydroxyl ion in the highly basic condition which will attack the surface silica shell of the sensor can destroy the specific binding site in the polymer. Consequently, pH 6.0 was selected as the appropriate condition and applied in subsequent experiments.

Effect of temperature

The effect of temperature (25-50 ℃) on the NGQDs@MIPs fluorescence signal has been investigated. As observed in Fig. S4c, 25 ℃ was the optimal temperature for the NGQDs@MIPs sensor system, and the fluorescence enhancement of the NGQDs@MIPs decreased with increasing temperature. The explanation for the phenomenon may be the non-radiative transition caused by the acceleration motion of molecules at high temperatures.

Effect of incubation time

To further estimate the accessibility of the recognition sites, the response time of the NGQDs@MIPs to warfarin was optimized. The solutions containing 0.1 mg mL^{-1} NGQDs@MIPs in the presence of 2.5 μM warfarin at pH 6.0 were prepared. Afterward, the fluorescence intensity was recorded in the range of 0-20 min (Fig. S4d). The signal

intensity of the sensor increased with time up to 4 min and almost remained constant. Therefore, 4 min was selected as the optimized response time for the detection of warfarin.

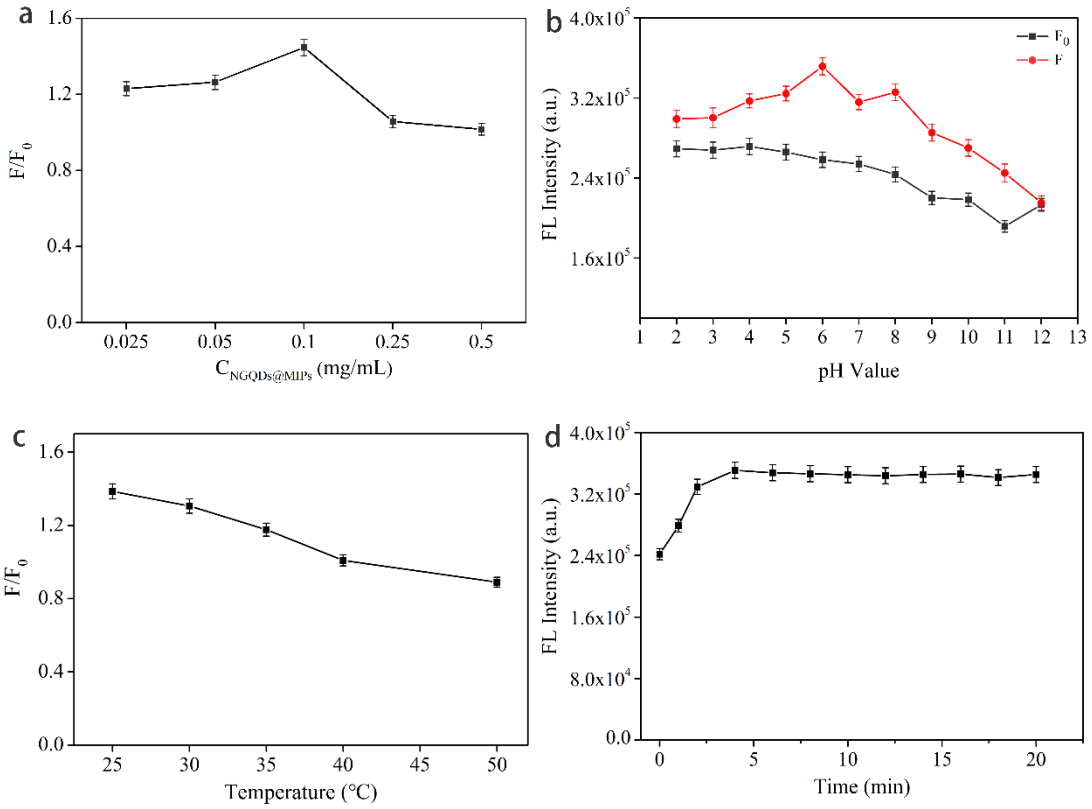

Fig. S4 (a) The effect of the concentration of sensor on the fluorescence response of MIPs to warfarin (2.5 μM); (b) The influence on fluorescence response of MIPs at pH 2-12; (c-d) Effect of temperature and time on the response of NGQDs@MIPs nanocomposite to warfarin solutions.

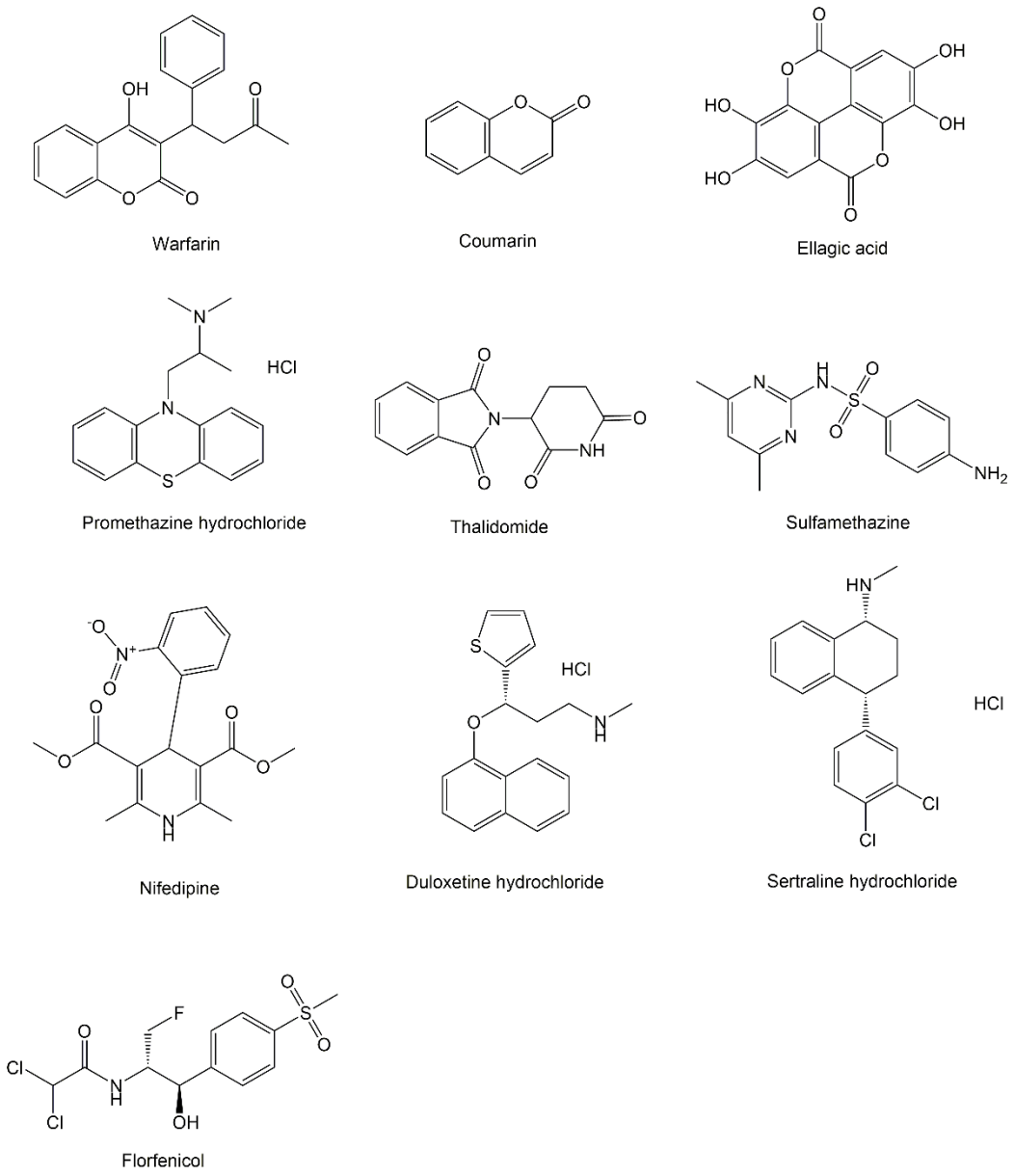

Fig. S5 The structure of warfarin and interfering substances.

Table S1 Comparison of Different Methods for the Quantification of Warfarin

Methods	Detector	LOD (μM)	Linear range (μM)	Recovery (%)	RSD (%)	Reference
HPLC-MS-MS	S-warfarin R-warfarin	0.003	-	95.7 92.5	< 7.3 < 6.5	6
MEKC-ESI-MS	warfarin	0.32	8-1.6	-	-	5
electrochemical sensor	warfarin	0.15	1-100	98.6-100.5	2.1-2.8	4
LFIA	warfarin	0.03	-	75.7-105.6	8.5-11.3	1
Fluorescence NGQDs@MIPs	warfarin	0.16	0.63-10	94.38-105.84	0.56-4.75	This work

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