Chromatin extracted from common carp testis as an economical and easily available adsorbent for ethidium bromide decontamination

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HIGHLIGHTS

- The inexpensive bio-adsorbent was prepared from common carp testis by-production.
- The bio-adsorbent was applied in EtBr decontamination.
- The maximum adsorption amount of EtBr by chromatin was up to 45.73 mg g\(^{-1}\), while the maximum adsorption amount of EtBr by activated carbon was only 0.46 mg g\(^{-1}\).
- The adsorption of EtBr by chromatin followed Pseudo-second-order kinetics and Langmuir isotherm model.

ABSTRACT

The waste of ethidium bromide (EtBr) used in the laboratory will bring a great burden to the environment, which need to be solved urgently. In the present paper, an efficient and inexpensive method for EtBr removal using chromatin extracted from common carp testis was investigated. The observation of fluorescence microscopy showed that chromatin had similar property to DNA for selective adsorption of EtBr. The results of batch adsorption showed that the removal efficiency of EtBr by chromatin exceeded 99% at pH 7.4 and 30 °C for 3 min with the EtBr concentration of 2 mg L\(^{-1}\) and the chromatin dosage of 0.5 g L\(^{-1}\), and the maximum adsorption amount of chromatin was 45.73 mg g\(^{-1}\). Further, the analysis of kinetic and isotherm suggested that the adsorption followed Pseudo-second-order kinetics and Langmuir isotherm model, and the calculated maximum theoretical adsorption amount of chromatin to EtBr was 48.08 mg g\(^{-1}\). According to thermodynamic analysis, chromatin adsorption of EtBr was a spontaneous process dominated by hydrogen bonding and van der Waals forces. This work will not only offer an adsorbent for EtBr decontamination, also provide a possibility for EtBr analogs removal.

1. Introduction

Ethidium bromide (EtBr) is a cationic dye, widely used as a DNA stain in molecular biology labs and pharmacology labs due to its 20-30-fold enhancement of fluorescent when intercalated in the region of double-stranded DNA base pair [1,2]. However, EtBr is highly mutagenic and carcinogenic based on the fact that EtBr causes mutations in bacterial gene fragments by interfering with DNA replication and has a significant mutagenic effect on Salmonella a typhimurium [3, 4, 5]. Even with such potential safety hazards, EtBr is still widely used in laboratories because...
of its low price and high sensitivity [6], and most laboratories discharge EtBr directly into the sewer without special treatment, which undoubtedly places a significant burden on the environment since EtBr is stable in the natural environment. In addition, direct discharge of EtBr waste can seep into groundwater for gardening, agriculture, washing and even drinking, which can have a direct impact on human health [7,8]. EtBr waste should urgently be treated as a hazardous pollutant, even though it is not yet classified as a possible human carcinogen by the International Agency for Research on Cancer (IARC).

Degradation and adsorption are the two most commonly used ways to remove EtBr from pollutants [9]. In almost laboratories, the common method to deal with EtBr waste is to degrade EtBr with sodium hypochlorite before discharge. Unfortunately, the degradation of EtBr by sodium hypochlorite is accompanied by many other derived compounds that have been found to be potentially more mutagenic than EtBr itself [10], which would pose an even greater risk to environment. Compared with degradation, adsorption is a more effective and potential method to remove environmental pollutants, which is more conducive to the further treatment of pollutants [11]. Previous reports have shown that some synthetic materials, such as montmorillonite [12] and copper nanoparticles [13], have good adsorption effects in the treatment of EtBr pollutant. However, these materials are cumbersome and expensive to synthesize; others require harsh conditions, including longer adsorption time and higher adsorption temperature, but fail to achieve high adsorption capacity, making them difficult to use as a fast, simple, efficient, and inexpensive adsorbent for the removal of EtBr pollutant. Massachusetts Institute of Technology, a practical example, spent $10, 650 to manage approximately 4.7 tons of EtBr waste generated in 2005 [14]. Therefore, there is an urgent need to develop an inexpensive and easily available adsorbent for the decontamination of EtBr pollutant.

Chromatin is a linear structure composed of nucleosome as the basic unit, which is connected by about 50 bp of linked DNA [15]. The nucleosome is composed of core of eight histones wrapped around 1.75 turns of 146 bp of DNA and locked by histone H1 in the opening of DNA double strand [16]. It is evident that chromatin possesses some of the properties of DNA, such as EtBr intercalated in DNA base pairs to form stable complex [17], so chromatin has a good potential as an adsorbent for the removal of EtBr from pollutants. It has been shown that the decontamination of pollutants can be accomplished by separating the complex formed by planar rigid substances intercalating into DNA, but the water solubility of DNA dictates it ultimately needs to be separated using external tools, such as magnetic beads [18, 19, 20], or loading onto certain substances that are insoluble in water for the adsorption of pollutants, sufficiently indicating that the complexity of this technique increases to the point where it is difficult to disseminate it widely. Chromatin, as a polymer of biological macromolecule, is expected to be a convenient adsorbent for the removal of EtBr pollutant as it can decontaminate the pollutants by centrifugation after adsorption target substances. There is no doubt that the cost of removal EtBr from pollutants can be reduced by preparing chromatin with easily available and cheap raw materials. Sperm contains a lot of chromatin, which makes it an ideal raw material for chromatin preparation. Cheap and high-quality chromatin can be obtained from common carp testis, a widely sourced and ubiquitous raw material for chromatin preparation. Common carp testis, a widely sourced and ubiquitous raw material for chromatin preparation. Common carp testis (10 g) was homogenized for the preparation of chromatin.

**2. Materials and methods**

**2.1. Materials and reagents**

Common carp testis with milky white appearance, were taken as raw material for preparation chromatin, stored at -70 °C. Common carp sperm DNA (csDNA) was extracted from common carp testis according to the Ojeda method [21]. EtBr was purchased from Shanghai Yuanye Biology Co., Ltd. Magnetic beads were bought from Enriching Biotechnology (Shanghai, China). Phosphate buffer, which was used to adjust the pH of the working solution, and DNA binding solution (2 M KCl, 0.01 M Tris, and pH 6.3) were prepared with distilled water.

**2.2. Instrumentation**

The IS-RDV3 temperature controlled shaker (crystal, USA) was used in EtBr removal assays. Freeze centrifuge (Sigma, Germany) was used in all centrifugation steps. Chromatin was dried in Alpha 1-4 LD plus freeze dryer (Christ, Germany). The concentration of EtBr was converted by Cary-60 UV-Spectrometer (Agilent Technology, USA). Chromatin before and after treatment with EtBr were characterized by FTIR spectroscopy (Spectrum Two, PerkinElmer). The fluorescence observations of csDNA, chromatin and magnetic beads were carried out with DM4B fluorescence microscope (Leica, Germany).

**2.3. Preparation of chromatin**

The procedure for extracting chromatin was based on the method presented by Artman [22]. Common carp testis (10 g) was homogenized with 100 mL of 1.5% sodium citrate solution by homogenizer, and the precipitate was retained after centrifugation at 12000 g for 15 min. Subsequently, the spermatozoid was prepared by homogenizing the precipitate with 100 mL of 0.25 M sucrose-1.5% sodium citrate solution, spread in 300 mL of 0.88 M sucrose-1.5% sodium citrate solution for 10 min. After centrifuging the spermatozoid at 12000 g for 15 min, the precipitate was obtained, and then washed three times with 20 mL of 0.05 M Tris-HCl-0.15 M NaCl (pH 7.5) to obtain pure nuclei. Further, the nuclei were broken by homogenizer with 100 mL of 0.01 M Tris-HCl (pH 8.0). Finally, the chromatin was obtained by centrifugation broken nuclei at 12000 g for 15 min, dried in a freeze-dryer and stored at room temperature.

**2.4. Batch adsorption assays**

Chromatin adsorption studies were performed with 4 mL of working solution at 30 °C in a temperature controlled shaker (300 rpm). Different initial EtBr concentration (1, 5, 50 mg L\(^{-1}\)) were used for adsorption experiments to investigate the effect of contact time (0.5, 1, 2, 10, and 20 min) on the removal efficiency of EtBr. Subsequently, adsorbent dosage (0.25, 0.5, 1.25, 2.5, 12.5 g L\(^{-1}\)), pH value (6.2–8.2) and temperature (4, 30, and 45 °C) were explored. All samples obtained from the adsorption experiments were centrifuged at 12000 g for 20 min. The supernatant was used for the determination of EtBr residues using a UV spectrophotometer with the wavelength of 480 nm [23,24], and the chromatin before and after adsorption were observed by DM4B fluorescence microscope. The removal efficiency (R, %) and the adsorption amount (q, mg g\(^{-1}\)) of EtBr were calculated using the following equations:
\[ R(\%) = \frac{C_0 - C_e}{C_0} \times 100 \]  

(1)

\[ q \text{ (mg} \cdot \text{g}^{-1}) = \frac{(C_0 - C_e) \times V}{W} \]  

(2)

where \( C_0 \) is the initial concentration of EtBr (mg L\(^{-1}\)), \( C_e \) is the residual concentration of EtBr (mg L\(^{-1}\)), \( V \) is the volume of the solution (L), and \( W \) is the amount of chromatin (g).

2.5. Removal of EtBr by activated carbon or csDNA

Removal of EtBr by activated carbon: 2.0 mg of activated carbon was immersed in a tube containing 1.0 mL of distilled water and incubated in a shaker at 300 rpm for 40 min to fully infiltrate the activated carbon. After centrifugation at 12000 g for 20 min, the precipitate was retained and mixed with 4 mL of EtBr solution (50 mg L\(^{-1}\)), and incubated in a shaker with 300 rpm at 30 °C for 20 min. After incubation, the mixture was centrifuged at 12000 g for 20 min and the supernatant was transferred to a new tube for detecting EtBr residue.

Removal of EtBr based on the intercalation with csDNA via magnetic beads separation: 0.5 mL of EtBr solution (100 mg L\(^{-1}\)) and 0.5 mL of csDNA solution (1 g L\(^{-1}\)) were transferred to a tube, incubated at 30 °C for 20 min while shaking with 300 rpm. Following the addition of magnetic beads (5.0 mg) and DNA binding buffer solution (1 mL), the mixture was further incubated for 45 min. After incubation, the tube was placed into magnetic separator for 5 min and the supernatant was transferred to a new tube for detecting EtBr residue. The magnetic beads obtained from above removal assays of EtBr were eluted three times with distilled water to remove free EtBr and then an appropriate amount of magnetic beads was dripped onto glass slide for observation with DM4B fluorescence microscope.

2.6. FTIR experiment

The chromatin was mixed with KBr in a ratio of 1:200 and ground into a powder in a mortar to make thin flakes. The FTIR absorption spectra in the range of 3000–500 cm\(^{-1}\) were scanned and recorded in FTIR using potassium bromide as the scanning background. In the same way, the FTIR spectra of EtBr and the chromatin treatment with EtBr were determined.
2.7. Reusability

The tubes containing 4 mL of 2 mg L\(^{-1}\) EtBr solution and 0.5 g L\(^{-1}\) chromatin were incubated at 300 rpm for 20 min at 30 °C. Followed by centrifugation at 12,000 rpm for 20 min, the supernatant was discarded and the precipitate was washed three times with ultrapure water, and then 4 mL of 2 mg L\(^{-1}\) EtBr solution was added to the tubes for repeat adsorption experiments. The supernatant collected at each step were used to determine the residual concentration of EtBr using UV spectroscopy.

3. Results and discussion

3.1. The characterisation of adsorption

3.1.1. FTIR analyses

As seen from Figure 1(c), chromatin had vibrational peaks in the range of 1300–1800 cm\(^{-1}\) resulting from DNA base pair interactions, and characteristic peaks in the range of 800–1300 cm\(^{-1}\) for DNA backbone vibrations caused by sugar and phosphate. 1541 cm\(^{-1}\) showed the characteristic A-T and C-G base pair vibration [25]. In addition, there was a clear infrared absorption characteristic peak of the carbonyl group of protein at 1657 cm\(^{-1}\) [26]. It was inferred that the sample of chromatin surely contained both protein and DNA components from the above spectroscopic results, which provided a possibility for EtBr removal, since EtBr could intercalate into DNA base pair spontaneously through π-π interactions and van der Waals forces, and about 2.5 base pairs could be intercalated by one EtBr molecule [27]. As seen from Figure 1(b), the chromatin intercalated with EtBr produced an infrared absorption peak at 2362 cm\(^{-1}\), which was consistent with the characteristic infrared peak of EtBr in Figure 1(a). This phenomenon indicated that EtBr was adsorbed by chromatin through intercalation.

3.1.2. Fluorescence microscope observation

Chromatin, a polymer composed of DNA and proteins, has properties similar to DNA. As presented in Figure 2, csDNA was adsorbed on the surface of magnetic beads without fluorescence under the fluorescence microscope, whereas fluorescent spots could be observed when the csDNA-EtBr complex was loaded into the surface of the magnetic beads, which could be explained by the fact that the csDNA, with no fluorescent

![Figure 3](image3.png)  
**Figure 3.** Effect of contact time on the removal efficiency of EtBr: a-e represented the initial EtBr concentration of 1, 2, 5, 10 and 50 mg L\(^{-1}\); chromatin dosage = 0.5 g L\(^{-1}\) and T = 30 °C.

![Figure 4](image4.png)  
**Figure 4.** Effect of initial concentration on the removal efficiency of EtBr: a-e represented the initial EtBr concentration of 1, 2, 5, 10 and 50 mg L\(^{-1}\); chromatin dosage = 0.5 g L\(^{-1}\) and T = 30 °C.

![Figure 5](image5.png)  
**Figure 5.** Effect of pH on the removal efficiency of EtBr at the chromatin dosage of 0.5 g L\(^{-1}\), [EtBr] = 50 mg L\(^{-1}\), T = 30 °C (P < 0.05).

![Figure 6](image6.png)  
**Figure 6.** Effect of chromatin dosage on the removal efficiency of EtBr: a-c: [EtBr] = 1, 5, 50 mg L\(^{-1}\); T = 30 °C.
microscopy, however, they were able to be observed secondary

3.2. Effect of adsorption parameters on EtBr removal ef

removal of EtBr from pollutants.

for EtBr, similar to the selective adsorption of EtBr by csDNA via its

fluorescence after adsorption of EtBr, which was obviously the same as the

fluorescence of the csDNA-EtBr complex, but different from their own

fluorescence. Therefore, chromatin also had strong selective adsorption

for EtBr, similar to the selective adsorption of EtBr by csDNA via its

intercalation, which was expected to be a special adsorbent for the

removal of EtBr from pollutants.

3.2. Effect of adsorption parameters on EtBr removal efficiency

3.2.1. Effect of contact time on EtBr removal efficiency

The contact time between chromatin and EtBr is an important factor

in adsorption process, and an appropriate adsorption time can increase

the removal efficiency of EtBr pollutant. Figure 3 showed that chromatin

rapidly removed EtBr within 2 min when the initial concentrations of

EtBr were lower than 2 mg L\(^{-1}\), also reached adsorption equilibrium

within 3 min even the initial concentrations of EtBr exceeded 2 mg L\(^{-1}\).

This could be interpreted by the fact that the rapid intercalation of EtBr

into DNA base pairs during the adsorption process, allowing the chromatin
to reach adsorption equilibrium in a very short period of time. Therefore,
the optimum adsorption time for EtBr pollutant removed by chromatin was 3 min, which was superior to the adsorption equilibrium
time of 20 min for activated carbon, 12 h for synthesized manganese (II) doped zinc (II) sulphide nanoparticles [28] and 15 min for nutraceutical industrial fennel seed spent [29].

3.2.2. Effect of initial EtBr concentration on EtBr removal efficiency

DNA in chromatin contains a limited number of base pairs with fixed

maximum adsorption sites. According to Figure 4, apparently, EtBr with the initial concentrations below 2 mg L\(^{-1}\) were almost entirely removed (R > 99%) by chromatin at the dosage of 0.5 g L\(^{-1}\), due to the ability of

DNA base pairs to completely accommodate low concentrations of EtBr. However, for the same chromatin dosage, the EtBr removal efficiency decreased as the initial concentration increased from 5 to 50 mg L\(^{-1}\), which might be due to the fact that the DNA binding sites in chromatin were close to saturation when the concentration of EtBr was too high. In general, the concentration of EtBr used in the laboratory for DNA staining is around 0.5 mg L\(^{-1}\) [12]. Thus, 0.5 g L\(^{-1}\) of chromatin was fully capable of removing laboratory-generated EtBr pollutant.

3.2.3. Effect of pH on EtBr removal efficiency

Changes in pH directly affect the amount of hydrogen and hydroxide

ions in the solution, altering the phosphate backbone structure of DNA and contributing to changes in the removal efficiency of EtBr by chromatin [30]. From Figure 5, the removal efficiency of EtBr gradually enhanced with increasing pH in the range of 6.2–7.4 at the initial EtBr concentration of 50 mg L\(^{-1}\) as well as the chromatin dosage of 0.5 g L\(^{-1}\), until the maximum removal efficiency of EtBr was reached at pH 7.4. However, the removal efficiency of EtBr gradually reduced with increasing pH above 7.4. This phenomenon could be explained that the electrostatic interaction between chromatin and EtBr was weakened since the amino groups of DNA were gradually positively charged in the presence of hydrogen ions when the pH of the solution was below 7.4, resulting in their binding being hindered [31]. In addition, the protonation of chromatin surface groups was weakened by the increase of hydroxide ions when the pH was higher than 7.4, causing interference with the adsorption of non-ionized EtBr by chromatin [32]. Therefore, the pH should be maintained at 7.4 to remove EtBr.

3.2.4. Effect of chromatin dosage on EtBr removal efficiency

The dosage of chromatin directly affects the removal efficiency of

EtBr because chromatin has adsorption sites that have a maximum

amount of adsorption for EtBr. Based on Figure 6, the removal efficiency of EtBr with the initial concentrations of 1, 5 and 50 mg L\(^{-1}\) was significantly enhanced when the chromatin dosage was raised from 0.25 to 12.5 g L\(^{-1}\), which meant the removal efficiency of EtBr was positively

| Table 1. The adsorption parameters of EtBr at 30 °C. |
|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Adsorbent      | Dosage (10\(^{-3}\) g) | Amount of EtBr (10\(^{-3}\) mg) | Removal efficiency (%) | Adsorption amount (mg g\(^{-1}\)) | Adsorption equilibrium time (min) | Extraction yield (%) |
| Chromatin      | 2.0             | 200             | 45.73           | 45.73           | 3              | 25.12           |
| csDNA          | 2.0             | 200             | 65.66           | 65.66           | 65             | 9.24            |
| Activated carbon | 2.0            | 200             | 0.46            | 0.46            | 20             | -               |
3.2.5. Effect of temperature on EtBr removal efficiency

In the adsorption reaction, temperature is a key factor. From Figure 7, when EtBr concentration was in the range of 1–50 mg L$^{-1}$, the removal efficiency of EtBr slightly decreased with increasing temperature, which might be related to the weakening of the interaction between chromatin and EtBr due to the enhancement of intermolecular thermal motion [33].

Based on the insignificant effect of temperature on the removal efficiency of EtBr, 30 °C, which is close to room temperature, was considered as the optimal temperature for EtBr removal.

3.3. The superiority of chromatin

Chromatin has an advantage in terms of adsorption capacity, yield and source. As shown in Table 1, under the same conditions, the removal efficiency of chromatin to EtBr was 45.73% and the adsorption amount was 45.73 mg g$^{-1}$, which was slightly lower than that of csDNA, because the removal efficiency of csDNA to EtBr was 65.66% and the adsorption amount was 65.66 mg g$^{-1}$. However, the extraction yield of chromatin was 2.7 times higher than that of csDNA [34], and the adsorption equilibrium time was only 1/20 of that of csDNA, which could compensate for the weak difference in the adsorption capacity completely. For the conventional activated carbon, its ability to adsorption of EtBr was relatively poor, not only in the removal efficiency of 0.46% and the adsorption amount of 0.46 mg g$^{-1}$, which was only 0.013 times of the adsorption amount of chromatin, but also in the adsorption equilibrium time of 20 min, which was 6.6 times of the equilibrium time of EtBr adsorption by chromatin. Moreover, although DNA extracted from common carp testis could effectively remove EtBr, the csDNA extraction process was more complex and stringent to the laboratory environment, especially the temperature, while chromatin preparation was generally performed at room temperature and can be easily manipulated. In addition, chromat, being a large molecule, was soluble in aqueous phase but was easily separated by centrifugation after adsorption of EtBr, while the extremely water-soluble csDNA molecular was so much smaller than that of chromatin that it cannot be separated easily after forming a complex with EtBr, except with the help of magnetic beads. What is even more surprising is that common carp testis, the raw material for the preparation of chromatin, wherever they are, could be easily obtained from the farm or market in a with being inexpensive. Therefore, chromatin had a strong superiority and was perfectly suitable as a cheap and efficient adsorbent for the removal of EtBr from pollutants.

3.4. Reusability of chromatin

Figure 8 showed that the chromatin continuously decontaminated 2 mg L$^{-1}$ EtBr working solution for five times and the removal efficiency remained above 90%. Therefore, chromatin was an inexpensive and reusable adsorbent with highly efficient for EtBr removal.

3.5. Adsorption kinetic, isotherm and thermodynamic of chromatin

3.5.1. Adsorption kinetic

To explore the mechanism of adsorption reaction, adsorption kinetics, including the Pseudo-first-order kinetics model and Pseudo-second-order kinetics model, was investigated. The Pseudo-first-order model equation is as follows [35]:

$$\ln(q_e - q_t) = \ln(q_e) - k_1 t$$  (3)

where $q_t$ is the adsorption amount of adsorbent at equilibrium (mg g$^{-1}$), $q_e$ is the adsorption amount of adsorbent at any time (mg g$^{-1}$), and $k_1$ is the kinetic adsorption rate constant at the pseudo-first-order (min$^{-1}$). The Pseudo-second-order model equation is as follows [36]:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e}$$  (4)

where $k_2$ is the kinetic adsorption rate constant at the Pseudo-second-order (g mg$^{-1}$ min$^{-1}$). The linear fit of the adsorption kinetics was illustrated in Figure 9.

The parameters of the adsorption kinetics were calculated from the fitted equations, listed in Table 2. The results showed that the Pseudo-second-order model ($R^2 \leq 0.991$) was more consistent with the adsorption process of EtBr than the Pseudo-first-order model ($R^2 \geq 0.999$) by comparing the correlation coefficients. Meanwhile, the theoretical value of $q_e$ calculated by the Pseudo-second-order model was close to the experimental value, while the Pseudo-first-order model was not. Therefore, it can be inferred that the adsorption of EtBr by chromatin was consistent with Pseudo-second-order model.

3.5.2. Adsorption isotherm

The adsorption isotherm reflects the interaction between the adsorbent surface and the adsorbent at a specific temperature for the adsorption equilibrium. Hence, the more commonly used Langmuir, Freundlich and Dubinin-Radushkevich isotherm model were chosen to describe the
adsorption isotherms of chromatin. The Langmuir isotherm model is defined by the following equation [37]:

\[
\frac{C_e}{q_e} = \frac{1}{K_L Q} + \frac{C_e}{Q}
\]  

(5)

where \( q_e \) is the equilibrium adsorption volume \( (\text{mg} \cdot \text{g}^{-1}) \), \( Q \) is the monolayer saturation adsorption volume \( (\text{mg} \cdot \text{g}^{-1}) \), \( C_e \) is the EtBr concentration in the working fluid at adsorption equilibrium \( (\text{mg} \cdot \text{L}^{-1}) \), and \( K_L \) is a constant related to the temperature or enthalpy change of the adsorption process. The Freundlich isotherm model is defined by the following equation [38]:

\[
\ln q_e = \ln K_F + \frac{1}{n} \ln C_e
\]  

(6)

where \( K_F \) and \( n \) are Freundlich constant related to the temperature or enthalpy change of the adsorption process. The Dubinin-Radushkevich isotherm model is defined by the following equation [39]:

\[
\ln q_e = \ln q_s + K_a \varepsilon
\]  

(7)

![Figure 10. Fitted isothermal model of chromatin adsorption of EtBr: Langmuir (a), Freundlich (b), Dubinin-Radushkevich (c).](image)

Table 2. Kinetic adsorption parameters of chromatin adsorption of EtBr at 30 °C.

| EtBr (mg L⁻¹) | Pseudo-first order | | Pseudo-second order | |
|--------------|--------------------|----|--------------------|----|
|              | \( K_1 \) (min⁻¹) | \( q_e \) (mg g⁻¹) | \( R^2 \) | \( K_2 \) (g mg⁻¹ min⁻¹) | \( q_e \) (mg g⁻¹) | \( R^2 \) |
| 1            | 3.08               | 2.72 | 0.991              | 7.50 | 2.01 | 1.000 |
| 2            | 1.66               | 1.16 | 0.455              | 1.96 | 4.03 | 1.000 |
| 5            | 1.56               | 14.01 | 0.872 | 0.27 | 9.28 | 1.000 |
| 10           | 2.26               | 31.98 | 0.984 | 0.22 | 15.48 | 0.999 |
| 50           | 1.66               | 24.30 | 0.951 | 0.18 | 45.67 | 1.000 |

![Figure 11. Thermodynamic fitting of \( \ln K \) and \(-1/T\).](image)

Table 3. Adsorption isotherm parameters of chromatins adsorption of EtBr.

| Model                      | Parameter                     | Parameter | Parameter | Parameter |
|----------------------------|-------------------------------|-----------|-----------|-----------|
|                            | \( T \) (°C)                  | \( K_L \) (L mg⁻¹) | \( q_s \) (mg g⁻¹) | \( R^2 \) |
| Langmuir                   | 4                             | 0.0208    | 48.08     | 0.960     |
|                            | 30                            | 0.0218    | 45.87     | 0.972     |
|                            | 45                            | 0.0220    | 45.45     | 0.974     |
|                            | \( T \) (°C)                  | \( n \)   | \( K_F \) (mg⁻¹ L¹/n g⁻¹) | \( R^2 \) |
| Freundlich                 | 4                             | 13.13     | 2.93      | 0.968     |
|                            | 30                            | 12.26     | 2.72      | 0.980     |
|                            | 45                            | 11.52     | 2.54      | 0.988     |
|                            | \( T \) (°C)                  | \( K_a \) (mol² kJ⁻¹) | \( q_s \) (mg g⁻¹) | \( R^2 \) | \( E \) (kJ mol⁻¹) |
| Dubinin-Radushkevich      | 4                             | \( -2 \times 10^{-8} \) | 20.13     | 0.804     |
|                            | 30                            | \( -2 \times 10^{-8} \) | 19.87     | 0.824     | 5.0       |
|                            | 45                            | \( -2 \times 10^{-8} \) | 19.13     | 0.811     |


where $K_{ad}$ is the isotherm constant with respect to Dubinin-Radushkevich; $q_i$ is the theoretical adsorption amount; and $\varepsilon$ is the Polanyi potential, which is calculated as follows [40]:

$$\varepsilon = RT\ln \left( 1 + \frac{1}{q_i} \right)$$

(8)

for the parameters in Dubinin-Radushkevich equation, the value of the average adsorption energy ($\bar{E}$) can be calculated, which represents the free energy used for the transfer of 1 mol of ions from the solution to the surface of the adsorbent, and it can be calculated from the following equation [41]:

$$E = - \frac{2\varepsilon}{\varepsilon - \varepsilon_0}$$

(9)

The linear fit of the adsorption isotherm was indicated in Figure 10. The parameters in Table 3 were obtained by data processing of the fitted equations for the adsorption isotherms. The results suggested that the adsorption process was more consistent with the Langmuir adsorption isotherm model as the correlation coefficients ($R^2$) of Langmuir model were all closer to 1 than those of Freundlich model, inferring that the adsorption sites on the chromatin surface were not homogeneous.

Meanwhile, the theoretical maximum adsorption capacity of chromatin on EtBr was 48.08 mg g$^{-1}$, which was relatively close to the actual maximum adsorption amount of 45.73 mg g$^{-1}$ obtained experimentally. In addition, the $E$ value of 5.00 kJ mol$^{-1}$ for chromatin adsorption of EtBr from Dubinin-Radushkevich model indicated that the reaction was physisorption [42]. Therefore, chromatin could be used as a potential adsorbent for the decontamination of EtBr from pollutants.

### 3.5.3. Adsorption thermodynamic

Thermodynamic parameters such as Gibbs free energy ($\Delta G$), enthalpy change ($\Delta H$) and entropy change ($\Delta S$), were used to further explain the adsorption mechanism with reference to the following equations:

$$\Delta G = - RT \ln K$$

(10)

$$\ln K = \ln K_0 + \frac{\Delta H}{RT}$$

(11)

$$\Delta G = \Delta H - T \Delta S$$

(12)

where $\Delta G$ is the Gibbs free energy (kJ mol$^{-1}$), $R$ is the gas constant, taken as 8.314 J mol$^{-1}$ K$^{-1}$; $K$ is the thermodynamic equilibrium constant of adsorption, which in this study is the model rate constant, $K_0$ is a constant; $\Delta H$ is the enthalpy change of adsorption (kJ). The linear fit of adsorption thermodynamic was shown in Figure 11.

According to Eq. (12), thermodynamic parameters calculated from the data of temperature-influenced factors were presented in Table 4. The results that the value of $\Delta G$ was negative and increased with the raising temperature inferred that chromatin adsorption of EtBr was a spontaneous and lowering the temperature favored the adsorption reaction. The absolute values of $\Delta G$ were smaller than 60 kJ mol$^{-1}$, indicating that the sorption process was physical, which was consistent with the results calculated by the Dubinin-Radushkevich isothermal model. Moreover, both $\Delta H$ and $\Delta S$ was negative, which indicated that the adsorption was exothermic reaction with hydrogen bonding and van der Waals forces as the dominant force.

### 4. Conclusion

Chromatin prepared from common carp sperm has been fully confirmed as a new potential adsorbent for the decontamination of EtBr pollutant through a series of experiments. Similar to the selective adsorption of EtBr by csDNA via its intercalation, chromatin also had strong selective adsorption for EtBr, which had been revealed by fluorescence microscope observation. The batch adsorption results revealed that chromatin reached adsorption equilibrium within 3 min at pH 7.4 and 30 °C. The removal efficiency of EtBr at 2 mg L$^{-1}$ was greater than 99% with the chromatin dosage of 0.5 g L$^{-1}$. EtBr was removed with the maximum efficiency of 45.73% at the excessive concentration of 50 mg L$^{-1}$ when the dosage of adsorbent was 0.5 g L$^{-1}$ within 3 min, and its maximum adsorption amount was 45.73 mg g$^{-1}$, while the maximum removal efficiency of EtBr by activated carbon under the same conditions was only 0.46% with the maximum adsorption amount was 0.46 mg g$^{-1}$ after 20 min of treatment. Moreover, the Pseudo-second-order kinetics and Langmuir isotherm model were evaluated for the adsorption process of EtBr into chromatin, and the maximum theoretical adsorption amount of chromatin to EtBr was converted to be 48.08 mg g$^{-1}$ by Langmuir isotherm model. Thermodynamic parameters demonstrated spontaneous adsorption of EtBr into chromatin, which was dominated by hydrogen bonding and van der Waals forces. Therefore, chromatin can be applied to decontaminate EtBr from pollutants and also give a possibility for the removal of EtBr analogs.

### Declarations

**Author contribution statement**

Jie Zhang: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Junsheng Li: Conceived and designed the experiments; Analyzed and interpreted the data.

Guoxia Huang & Liujuan Yan: Contributed reagents, materials, analysis tools or data.

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**Data availability statement**

Data will be made available on request.

**Declaration of interests statement**

The authors declare no conflict of interest.

**Additional information**

No additional information is available for this paper.

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