REMOVAL OF ORGANIC POLYCYCLIC COMPOUNDS FROM WATER SOLUTIONS WITH A MAGNETIC CHITOSAN BASED SORBENT BEARING COPPER PHTHALOCYANINE DYE

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Abstract—Magnetic chitosan gel particles, bearing covalently immobilized copper phthalocyanine dye ("magnetic blue chitosan"), were prepared and used for the isolation and/or removal of polycyclic dyes from water solutions and suspensions. Binding of these polycyclic dyes occurs by a chemical equilibrated and saturatable mechanism, following the Langmuir adsorption model. The values of maximum uptake (maximum adsorption capacity) were calculated. The bound dyes can be eluted from the sorbent with methanol, methanol–conc. ammonia solution (50:1, v/v) and 10% acetic acid.

Key words — magnetic particles, chitosan, phthalocyanine dye, polycyclic compounds, adsorption capacity, magnetic blue chitosan

NOMENCLATURE

\[ b = \text{constant [dm}^3\text{mg}^{-1}] \text{ or [l/mg]} \]
\[ C_e = \text{equilibrium liquid-phase concentration [mg dm}^{-3}] \text{ or [mg/l] for calculations based on the settled volume of the magnetic sorbent, or [mg g}^{-1}] \text{ for calculations based on dry weight of the sorbent}} \]
\[ q_m = \text{equilibrium solid phase concentration [mg cm}^{-2}] \text{ or [mg/ml] for calculations based on the settled volume of the magnetic sorbent, or [mg g}^{-1}] \text{ for calculations based on dry weight of the sorbent}} \]
\[ Q = \text{maximum adsorption capacity of the sorbent [mg cm}^{-2}] \text{ or [mg/ml] for calculations based on the settled volume of the magnetic sorbent, or [mg g}^{-1}] \text{ for calculations based on dry weight of the sorbent}} \]

INTRODUCTION

Many organic polycyclic compounds have mutagenic and carcinogenic properties. They are found in the environment and also in some foods as a result of their thermal treatment (Davidek et al., 1990). It is important to monitor their content in various matrices and therefore suitable analyses have to be performed.

Recently a simple and useful procedure for preconcentration of organic compounds having three or more fused rings in their structure has been described. These compounds are adsorbed on blue cotton, i.e. on cotton bearing covalently immobilized copper phthalocyanine dye (usually in the form of C.I. Reactive Blue 21). The adsorbed compounds can be easily eluted from the sorbent with a mixture of methanol and concentrated ammonia (50:1, v/v). The complete information on this topic can be found in an excellent review article written by Hayatsu (1992).

For trapping of mutagens and carcinogens or other organic compounds with planar structure in suspensions or for certain biological and medical experiments magnetic sorbents carrying copper phthalocyanine would be useful. Recently Povey and O’Neill (1990) have prepared magnetic polyethyleneimine microcapsules containing the above mentioned dye; they have used these microcapsules for trapping and biomonitoring of mutagens having a planar molecular structure, such as benzo[a]pyrene and its metabolites, within the gastrointestinal cavity.

For many purposes (e.g. for waste water treatment and analysis) cheap and easily preparable magnetic sorbents carrying copper phthalocyanine are needed. In this paper preparation and evaluation of such a sorbent, based on magnetic chitosan matrix (magnetic blue chitosan), is described.

EXPERIMENTAL

Materials

Chitosan (medium mol. wt) was from Fluka, Switzerland. Reactive copper phthalocyanine dye (Ostatin turquoise V-G; C.I. Reactive Blue 21) was from Spolek pro chemickou a hutní výrobu, Ústí nad Labem, Czech Republic. Iron(II, III) oxide (magnetite) was obtained from Aldrich, U.S.A. Glutaraldehyde (25% solution) was from Serva, Germany. Congo red (mol. wt 696.7, purity 50%) and safranin O (mol. wt 350.8, purity 98%) were from Sigma, U.S.A. Acidine orange (mol. wt 301.8, purity cca 75%) was from Merck, Germany. Neutral red (mol. wt 288.8, purity cca 75%), crystal violet (mol. wt 408.0, purity cca 75%), ethanolamine and common chemicals were from Lachema, Czech Republic. Strong permanent magnets of the Ormacon type, used for the separation of magnetic particles, were obtained from Dr Z. Blažek, Department of Magnets,
National Research Institute of Materials, Prague, Czech Republic.

**Preparation of magnetic chitosan**

Chitosan (1 g) was dissolved in 20 ml of 5% acetic acid. After complete dissolution (approx 3 h) 4 g of magnetite were added to the viscous solution. After thorough mixing 35 ml of 6% sodium hydroxide were added and the gel formed was thoroughly mixed with the hydroxide solution to neutralize the acetic acid present inside the gel particles. The suspension was allowed to stand under occasional stirring for 3-4 h. The gel was then homogenized in a mixer to form small particles of magnetic chitosan. The particles were thoroughly washed with water until the pH of the washings was the same as that of water.

The suspension of magnetic chitosan particles (total vol. 130 ml) was mixed with 15 ml of 1 M phosphate buffer, pH 7.4 and then 5 ml of 25% glutaraldehyde were added. The cross-linking was allowed to proceed at ambient temperature under occasional stirring for 18 h. The particles were then washed with water. The remaining free aldehyde groups were blocked with ethanolamine (2 ml added to 130 ml of the magnetic chitosan suspension). After overnight incubation the magnetic chitosan particles were thoroughly washed with water and stored at 4°C. The dry weight of 1 ml of the settled magnetic chitosan particles was 64 mg.

![Chemical structures](image_url)

**Acridine orange**

![Chemical structures](image_url)

**Congo red**

![Chemical structures](image_url)

**Crystal violet**

![Chemical structures](image_url)

**Neutral red**

![Chemical structures](image_url)

**Safranin O**

Fig. 1. Structures of dyes used.
Removal of organic polycyclic compounds

Determination of sorption capacity of the magnetic blue chitosan

To the suspensions of blue magnetic chitosan (200 μl; the settled volume of the sorbent was 50 μl) in 15-ml polystyrene test tubes 8.8 ml of water were added. Then 0.01-1.0 ml portions of stock water solutions (1 mg/ml) of polycyclic dyes tested were added and the total volume of the suspension was made up to 10.0 ml with water. In the same manner water solutions of the tested dyes, used for the construction of the calibration curves, were prepared; instead of 200 μl of blue chitosan suspension, 200 μl of water were used. The suspensions were mixed for 4 h at 20°C. Then the magnetic blue chitosan particles were separated from the suspension using a permanent magnet held on the test-tube wall and the clear supernatants were used for the determination of absorbances. The concentration of free (unbound) dye in the supernatant was determined from the calibration curve and the amount of bound dye was calculated by difference.

Elution of bound dyes

To the suspensions of blue magnetic chitosan (500 μl; the settled volume of the sorbent was 125 μl) in 15-ml polystyrene test tubes 1 ml of water solutions of the tested dyes (1 mg/ml) were added. After a 4 h incubation period the dye solution was poured off and the sorbent was washed six times with 2 ml of water. The solutions were combined together and the concentration and the total amount of nonbound dye were calculated from the calibration curve. Appropriate eluent solution (5 ml) was then added to the magnetic blue chitosan and after 15 min mixing the solution was poured off. The concentration and the total amount of the released dye was calculated from the calibration curve (the dye tested and the eluent solution were used for its preparation). The elution efficiency was expressed in percentage, calculated from the amounts of bound and released dye.

RESULTS

Entrapment of fine particles of magnetite into the structure of cross-linked chitosan led to the formation of magnetically responsible gel particles with free hydroxyl groups. The magnetic particles were easily removed from the solutions or suspensions with the aid of a small permanent magnet. Hydroxyl groups can be utilized for the immobilization of reactive textile dyes, in a similar manner as described previously for various polysaccharide derivatives (Stellwagen, 1990; Šafařík, 1990).

Magnetic chitosan particles bearing copper phthalocyanine dye (magnetic blue chitosan) could adsorb various organic compounds with polycyclic structure. Equilibrium sorption isotherms of acridine orange, congo red, crystal violet, neutral red and safranine O (see Fig. 1 for their structures) by magnetic blue chitosan with no pH control are shown in Fig. 2. Sorption isotherms represent the equilibrium distribution of dye molecules between the aqueous and solid phases, when the dye concentration increases. The binding of the dye to the surface of magnetic blue chitosan was then considered in terms of the Langmuir isotherm, the following linearized form being used:

\[
\frac{C_{eq}}{q_{eq}} = \frac{1}{b \cdot Q} + \frac{C_{eq}}{Q}
\]

Preparation of magnetic blue chitosan

One hundred ml of magnetic chitosan particles suspension (supernatant:sediment = 1:1) was mixed with 2 g of Ostazin turquoise V-G (C.I. Reactive Blue 21) and 6 g of sodium chloride. The suspension was warmed to 70°C and 15 min later 5 g of anhydrous sodium carbonate were added. The suspension was stirred at 70°C for 3 h and then the mixture was left overnight at ambient temperature without mixing. The magnetic blue chitosan particles were thoroughly washed with water and the remaining free colour was removed using an extraction with methanol in a Soxhlet apparatus. The extracted particles were then repeatedly washed with methanol—conc. NH₄OH mixture (50/1, v/v) and with dimethyl sulfoxide until only faint blue washings were obtained. The washed magnetic blue chitosan particles were stored in water at 4°C. The dry weight of 1 ml of the settled magnetic blue chitosan particles was 66 mg.

Fig. 2. Equilibrium sorption isotherms of acridine orange (●), congo red (▲), crystal violet (●), neutral red (■) and safranine O (▼) using magnetic blue chitosan as sorbent.

Fig. 3. Langmuir transformation of equilibrium sorption isotherms (magnetic blue chitosan used as sorbent). The symbols are the same as in Fig. 2.
Table 1. Coefficients of Langmuir transformations ($m$, $n$), maximum adsorption capacities ($Q$) and Langmuir constants ($b$). Magnetic blue chitosan was used as sorbent. $Q^*$ and $Q'$ are maximum adsorption capacities calculated for pure dyes

<table>
<thead>
<tr>
<th>Dye</th>
<th>$y = m \cdot x + n$</th>
<th>$Q$ (mg/ml) (mg/g)</th>
<th>$Q^*$ (mg/ml) (μmol/g)</th>
<th>$Q'$ (mg/ml) (μmol/g)</th>
<th>$b$ (l/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidine orange</td>
<td>0.248 (0.016)</td>
<td>4.032 (61.08)</td>
<td>10.02 (151.8)</td>
<td>0.451 (0.451)</td>
<td></td>
</tr>
<tr>
<td>Congo red</td>
<td>0.441 (0.029)</td>
<td>2.267 (34.34)</td>
<td>1.626 (24.65)</td>
<td>0.370 (0.370)</td>
<td></td>
</tr>
<tr>
<td>Crystal violet</td>
<td>0.211 (0.014)</td>
<td>4.739 (71.80)</td>
<td>8.711 (132.0)</td>
<td>0.249 (0.249)</td>
<td></td>
</tr>
<tr>
<td>Neutral red</td>
<td>0.322 (0.021)</td>
<td>3.106 (47.06)</td>
<td>2.329 (53.28)</td>
<td>0.604 (0.902)</td>
<td></td>
</tr>
<tr>
<td>Safranin O</td>
<td>0.366 (0.024)</td>
<td>2.732 (41.39)</td>
<td>2.623 (39.74)</td>
<td>0.434 (0.434)</td>
<td></td>
</tr>
</tbody>
</table>

The values in upper lines (without parentheses) are calculated using the settled volume of the magnetic sorbent; the values in parentheses are calculated using the dry weight of the sorbent.

where $q_{eq}$ is the amount of dye adsorbed per unit of sorbent ("bound dye"), $C_{eq}$ is the concentration of dye remaining in solution at equilibrium ("free dye"), $Q$ is maximum adsorption capacity (number of mol or g of dye adsorbed per unit of sorbent forming a continuous monolayer on sorbent surface) and $b$ is a constant.

The isotherms follow the typical Langmuir adsorption pattern, as shown by the linear transformation (Fig. 3). The Langmuir model is based on four basic assumptions, viz. (Ruthven, 1984):

1. Sorbate (in this case polymeric dye molecules) is chemically adsorbed at a fixed number of well-defined sites
2. Each site can hold one sorbate molecule
3. All sites are energetically equivalent
4. There is no interaction between molecules adsorbed on neighbouring sites.

The results show that the sorption of organic polycyclic molecules by blue magnetic chitosan is a chemical, equilibrated and saturable mechanism. Thus, adsorption increases when the initial dye concentration rises, as long as binding sites are not saturated. Linear transformation allows the calculation of the maximum adsorption capacity $Q$ (see Table 1).

It is well known that some dyes (e.g. the direct dyes) can strongly interact with various polysaccharides. To distinguish if the sorption of the dye was caused by the presence of the immobilized phthalo-cyanine dye or if the sorption on the polysaccharide matrix took place, the sorption experiments were carried out with the magnetic cross-linked chitosan only. As can be seen from Fig. 4, congo red (a typical example of the direct dye) adsorbed strongly on the cross-linked chitosan. On the contrary, crystal violet exhibited almost no sorption. Maximum adsorption capacities $Q$, calculated from the linear transformations (Fig. 5), are shown in Table 2.

Adsorbed dyes can be eluted from the magnetic blue chitosan particles with methanol, methanol containing conc. NH$_4$OH (50/1, v/v) or with diluted

![Fig. 4. Equilibrium sorption isotherms of acridine orange (○), congo red (△), crystal violet (○), neutral red (□) and safranin O (▼) using unmodified magnetic chitosan as sorbent.](image)

![](image)

![Fig. 5. Langmuir transformation of equilibrium sorption isotherms (unmodified magnetic chitosan used as sorbent). The symbols are the same as in Fig. 4.](image)
Table 2. Coefficients of Langmuir transformations \((m, n)\), maximum adsorption capacities \((Q)\) and Langmuir constants \((b)\). Unmodified magnetic chitosan was used as sorbent. \(Q^+\) and \(Q^-\) are maximum adsorption capacities calculated for pure dyes.

<table>
<thead>
<tr>
<th>Dye</th>
<th>(y = m \cdot x + n)</th>
<th>(m)</th>
<th>(n)</th>
<th>(Q_{mg/ml}) (mg/g)</th>
<th>(Q_{mg/ml}) (mg/g)</th>
<th>(Q^+_{\mu mol/ml}) (μmol/g)</th>
<th>(Q^-_{\mu mol/ml}) (μmol/g)</th>
<th>(b) (l/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidine orange</td>
<td>2.196</td>
<td>48.82</td>
<td></td>
<td>0.455</td>
<td>0.341</td>
<td>1.130</td>
<td>0.045</td>
<td>0.045</td>
</tr>
<tr>
<td></td>
<td>(0.141)</td>
<td>(3.124)</td>
<td></td>
<td>(7.109)</td>
<td>(5.328)</td>
<td>(17.66)</td>
<td>(0.045)</td>
<td></td>
</tr>
<tr>
<td>Congo red</td>
<td>0.360</td>
<td></td>
<td>0.046</td>
<td>2.778</td>
<td>1.389</td>
<td>1.994</td>
<td>8.999</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.023)</td>
<td>(0.003)</td>
<td></td>
<td>(43.41)</td>
<td>(21.70)</td>
<td>(31.16)</td>
<td>(8.999)</td>
<td></td>
</tr>
<tr>
<td>Crystal violet</td>
<td>102.9</td>
<td>12.61</td>
<td></td>
<td>0.010</td>
<td>0.008</td>
<td>0.020</td>
<td>7.930</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(6.586)</td>
<td>(0.807)</td>
<td></td>
<td>(0.16)</td>
<td>(0.125)</td>
<td>(0.312)</td>
<td>(7.930)</td>
<td></td>
</tr>
<tr>
<td>Neutral red</td>
<td>1.408</td>
<td>5.254</td>
<td></td>
<td>0.710</td>
<td>0.533</td>
<td>1.846</td>
<td>0.268</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.090)</td>
<td>(0.336)</td>
<td></td>
<td>(11.09)</td>
<td>(8.328)</td>
<td>(28.84)</td>
<td>(0.268)</td>
<td></td>
</tr>
<tr>
<td>Safranin O</td>
<td>2.310</td>
<td>87.54</td>
<td></td>
<td>0.433</td>
<td>0.416</td>
<td>1.186</td>
<td>0.026</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.148)</td>
<td>(5.603)</td>
<td></td>
<td>(6.766)</td>
<td>(6.500)</td>
<td>(18.53)</td>
<td>(0.026)</td>
<td></td>
</tr>
</tbody>
</table>

The values in upper lines (without parentheses) are calculated using the settled volume of the magnetic sorbent; the values in parentheses are calculated using the dry weight of the sorbent.

Table 3. Efficiency of eluting solvents

<table>
<thead>
<tr>
<th>Dye</th>
<th>CH₃OH</th>
<th>CH₃OH–NH₂OH</th>
<th>CH₃COOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidine orange</td>
<td>42.4</td>
<td>36.8</td>
<td>60.9</td>
</tr>
<tr>
<td>Congo red</td>
<td>14.8</td>
<td>88.6</td>
<td>0</td>
</tr>
<tr>
<td>Crystal violet</td>
<td>54.6</td>
<td>56.8</td>
<td>94.1</td>
</tr>
<tr>
<td>Safranin O</td>
<td>38.8</td>
<td>26.8</td>
<td>68.3</td>
</tr>
</tbody>
</table>

The values are recorded in °C.

acetic acid. In Table 3, the recoveries of tested dyes are shown.

Separation of polyaromatic compounds, using magnetic blue chitosan particles, can be also performed in suspensions. Magnetic sorbent can be attracted with the aid of a magnet to the vertical wall of the test-tube or flat culture vessel, leaving the nonmagnetic particles present in the suspension to sediment.

**DISCUSSION**

As can be seen from the results, magnetic blue chitosan can be prepared in the simple way and at low cost. It can be used for simple removal and/or concentration of various polyaromatic compounds. Due to the magnetic properties of the sorbent, it can be also used in suspensions. It is a great advantage in comparison with traditional batch or column concentration and separation techniques.

Immobilized phthalocyanine dyes show high selectivity toward polycyclic aromatic compounds, especially those having three or more fused rings in their structure. These compounds are commonly planar in their molecular form. Consequently, it is conceivable that they can form face-to-face hydrophobic complexes with copper phthalocyanine moiety, which has a large planar surface in the molecule (Hayatsu, 1992). The planarity of crystal violet molecule is probably also responsible for the binding of this dye, even in the absence of fused-ring molecular structure.

The polysaccharide matrix used for the preparation of the magnetic particles, i.e. chitosan, can also react with direct dyes. Congo red, a typical example, adsorbed both on magnetic blue chitosan and unmodified magnetic chitosan. Comparing the values of maximum adsorption capacity \(Q\) for both sorbents (Tables 1 and 2) it can be concluded that no interaction with the immobilized phthalocyanine dye took place; on the contrary this immobilized ligand blocked parts of the chitosan chains and smaller amount of congo red could bind to the magnetic blue chitosan.

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**REFERENCES**


