

INTERACTION OF TRICYCLIC DRUGS WITH COPPER PHTHALOCYANINE DYE IMMOBILIZED ON MAGNETIC CARRIERS

M. Safarikova & I. Safarik

Laboratory of Biochemistry and Microbiology, Institute of Landscape Ecology, Na Sadkach 7, 370 05 Ceske Budejovice, Czech Republic

INTRODUCTION: During the last decades, the abuse of various drugs has increased considerably. This situation resulted in the development of different procedures for the detection and determination of the target drugs in various matrices, such as urine, serum, saliva, hair etc.

In many cases a two-stage process is generally employed for drugs of abuse testing in urine or other body fluids. At first, an appropriate screening test is used to differentiate between negative and positive samples. Various types of immunoassays can be used as screening tests. Alternatively, simple chromatographic procedures such as thin-layer chromatography (TLC) can be used for the same purpose. Results of positive screening tests are confirmed by a second technique such as gas or high-performance liquid chromatography with mass spectrometry detection (GC-MS, HPLC-MS).

The sensitivity of the instrumental techniques used for the confirmation and detailed analysis may not be sufficient to handle the samples directly, without preconcentration of the target compound(s). That's why the targets present in urine or other body fluids are usually extracted before chromatography is applied. There are two basic types of extraction procedures used namely liquid-liquid extraction (LLE) and solid-phase extraction (SPE). At present, considerable attention is being paid to SPE, mainly in the minicolumn format. Various types of adsorbents can be used, ranging from standard C18 silica to highly specific immunoaffinity adsorbents.

The separation and preconcentration of an analyte from large volumes of solution can take a lot of time using standard column SPE. Another procedure has also been developed for SPE, based on the use of magnetic or magnetizable adsorbents [1]. The procedure called magnetic solid-phase extraction (MSPE), based on the adsorption of the target analyte(s) on relatively small amount of magnetic specific adsorbent, enables to handle liter volumes of samples. In this procedure magnetic adsorbent is added to a solution or suspension containing the target analyte. The analyte is adsorbed on to the magnetic adsorbent

and then the adsorbent with adsorbed analyte is recovered from the suspension using an appropriate magnetic separator. The analyte is consequently eluted from the recovered adsorbent and analyzed.

Tricyclic drugs (named after their chemical structure) form a very important group of drugs, having antidepressant, antihistaminic, antiparkinsonic etc. effects. The major effects of tricyclic antidepressants are sedation, anticholinergic effects and the ability to inhibit the uptake of noradrenaline and/or serotonin into presynaptic neurons. The major clinical use of the tricyclic antidepressants is the reduction of the symptoms of depression. Concern about these medications is a result of their extensive list of side effects and drug interactions, and also because of the ability of these medications to be misused, e.g. by a depressed and suicidal patient. Intoxication with tricyclic antidepressants, e.g., amitriptyline, may cause severe complications such as cardiac arrhythmia. Even under intensive care conditions, 2-3% of intoxicated patients still die.

Adsorbents having affinity for tricyclic drugs would be useful in the development of specific preconcentration procedures. Although tricyclic drugs usually have side chains in their molecules that could affect their planar molecular structure, specific interactions with copper phthalocyanine derivatives can be expected. Immobilized derivatives of copper phthalocyanines have found many applications in analytical, bioanalytical and environmental chemistry [2]. They are used for the separation and isolation of planar organic compounds, such as polyaromatic hydrocarbons and their derivatives with three or more fused aromatic rings [2] and triphenylmethane dyes [3]. The planar target compounds can form face-to-face hydrophobic complexes with copper phthalocyanine moiety, which has a large planar surface in the molecule.

In this work reactive copper phthalocyanine dye immobilized to silanized magnetite particles ("blue magnetite") was used as a specific magnetic adsorbent for the study of interactions with several tricyclic drugs.

METHODS: Blue magnetite (silanized magnetite particles with immobilized reactive copper phthalocyanine dye) was prepared as described recently (3). The tricyclic drugs amitriptyline (3-(10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5-ylidene)-N,N-dimethyl-1-propanamine; for structure formula see Fig. 1), chlorprothixene (3-(2-chloro-9H-thioxanthen-9-ylidene)-N,N-dimethyl-1-propanamine), diethazine (Deparkin; N,N-diethyl-10H-phenothiazine-10-ethanamine), bisulepin (Dithiaden; N,N-dimethyl-3-thienol[2,3-c][2]benzothiepin-4(9H)-ylidenepropylamine hydrochloride; for structure formula see Fig. 1), prochlorperazine (2-chloro-10-[3-(4-methyl-1-piperazinyl)propyl]-10H-phenothiazine), thioridazine (10-[2-(1-methyl-2-piperidiny)ethyl]-2-(methylthio)-10H-phenothiazine) and dothiepin (Prothiaden; 3-dibenzo[b,e]-thiepin-11(6H)-ylidene-N,N-dimethyl-1-propanamine) were obtained from Leciva, Czech Republic. The drug action is shown in Table 1.

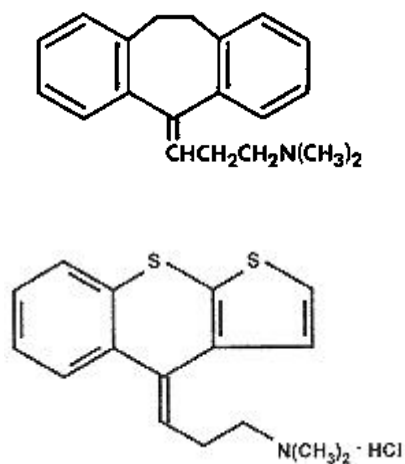


Fig. 1: Chemical structures of amitriptyline (top) and bisulepin (bottom).

The adsorption characteristics of the blue magnetite were studied in the following way. Suspensions of blue magnetite (200 μL ; the settled volume of the adsorbent was 50 μL) in 15-mL test tubes were mixed with 8.8 mL of water. Then 0.01 - 1.0 mL portions of stock water solutions (1 mg/mL) of tricyclic drugs 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 drugs, used for the construction of the calibration curves, were prepared (instead of 200 μL of blue magnetite suspension 200 μL of water

were used). The suspensions were mixed for 1 h at room temperature. Then the blue magnetite particles were separated from the suspension using a magnetic separator (MPC-1 or MPC-6, Dynal, Norway) and clear supernatants were used for the spectrophotometric measurement. The concentration of free (unbound) drug in the supernatant was determined from the calibration curve and the amount of bound drug was calculated by the difference.

The elution of the adsorbed drugs from the blue magnetite was performed using methanol as an elution agent.

RESULTS: Immobilization of reactive copper phthalocyanine dye to the silanized magnetite particles leads to the formation of the magnetically responsible affinity adsorbent with the specificity towards organic polyaromatic compounds with planar molecular structure. It was shown in preliminary experiments that the tested tricyclic drugs interacted with blue magnetite. The adsorption of tricyclic drugs to the adsorbent reached equilibrium in ca 30 min. In the following experiments the sorption of the tested drugs took place for 60 min.

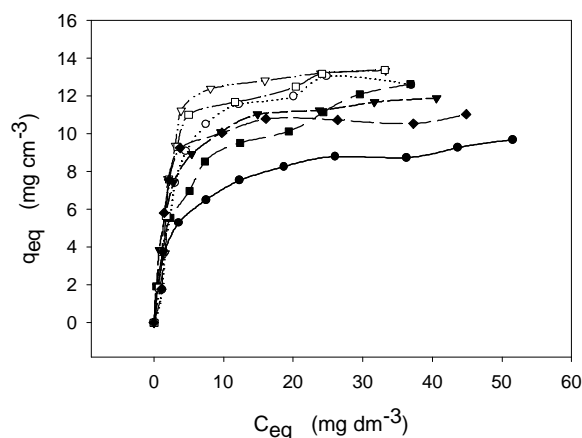


Fig. 2: Equilibrium adsorption isotherms of amitriptyline (●), bisulepin (○), chlorprothixene (▽), diethazine (■), dothiepin (▼), prochlorperazine (□) and thioridazine (◆) using blue magnetite as an adsorbent. C_{eq} – equilibrium liquid-phase concentration of the unadsorbed (free) drug (mg dm^{-3}); q_{eq} – equilibrium solid-phase concentration of the adsorbed drug (drug uptake) (mg cm^{-3}).

Equilibrium sorption isotherms for unbuffered water solutions are shown in Fig. 2. The tricyclic drugs adsorbed from water solution to immobilized phthalocyanine in a specific way. In a control

experiment when silanized magnetite was used as an adsorbent very low non-specific adsorption was observed.

The binding of tricyclic drugs to immobilized copper phthalocyanine was evaluated according to a system of classification of the solution adsorption isotherms described by Giles et al. (see Ref. 3). From Fig. 2 it can be seen that the isotherm follow the typical Langmuir adsorption pattern and belong to the class L2. It was also confirmed by the linear transformation of the data used for the construction of the adsorption isotherm (see Fig. 3).

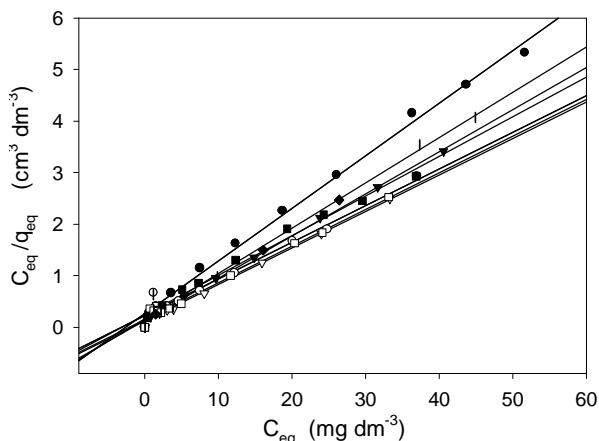


Fig. 3: Langmuir transformation of equilibrium adsorption isotherms for tricyclic drugs. The symbols and legends are the same as in Fig. 2.

Maximum adsorption capacities Q were calculated for all the drugs tested, using the linearized form of the Langmuir isotherm:

$$C_{eq}/q_{eq} = 1/b.Q + C_{eq}/Q \quad (1)$$

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

The obtained values are shown in Table 1.

The adsorbed tricyclic drugs have been eluted with methanol; approximately 40 – 50 % of bound drugs were eluted.

The presence of molecular interaction between a drug and copper phthalocyanine derivative can be observed using a spectroscopy study. Fig. 4 shows visible absorption spectra of copper phthalocyanine (spectrum 1), amitriptyline (spectrum 2) and their

mixture (spectrum 3). A significant shift of the phthalocyanine peak at 666 nm to a longer wavelength (difference ca 11 nm) is seen, indicating such interactions.

Table 1. Maximum adsorption capacities of the blue magnetite for the tricyclic drugs. Q is calculated using the settled volume of the magnetic adsorbent (mg mL^{-1}) while Q' is calculated using the dry weight of the adsorbent (mg g^{-1}).

| Drug | Drug action | Q | Q' |
|------------------------|-----------------|------|------|
| Amitriptyline | Antidepressant | 9.8 | 30.1 |
| Chlorprothixene | Neuroleptic | 14.2 | 43.6 |
| Diethazine (Deparkin) | Antiparkinsonic | 13.0 | 39.9 |
| Bisulepin (Dithiaden) | Antihistaminic | 14.0 | 43.0 |
| Prochlorperazine | Neuroleptic | 14.1 | 43.3 |
| Dothiepin (Prothiaden) | Antidepressant | 11.4 | 35.0 |
| Thioridazine | Neuroleptic | 11.4 | 35.0 |

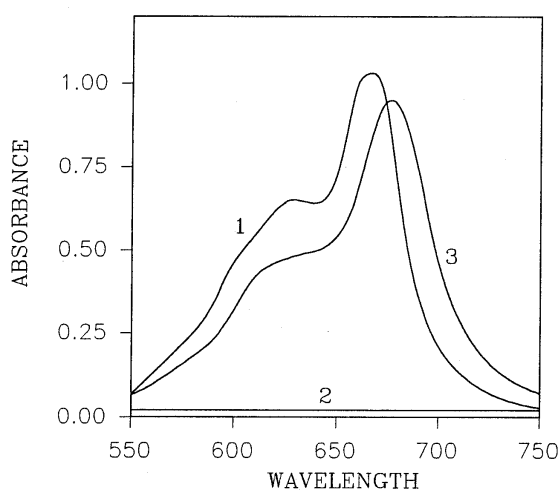


Fig. 4: Visible absorption spectra of copper phthalocyanine (spectrum 1), amitriptyline (spectrum 2) and their mixture (spectrum 3).

DISCUSSION & CONCLUSIONS: As can be seen from the results, immobilized copper phthalocyanine derivative specifically interacts with all tricyclic drugs tested. It was found that other drugs with more fused rings and a side chain in the molecular structure can also be adsorbed to the mentioned adsorbent. For example lisuride (N'-[(8 α)-9,10-didehydro-6-methylergolin-8-yl]-N,N-diethylurea) was adsorbed with the maximum adsorption capacity $Q = 14.6 \text{ mg mL}^{-1}$ (other data not shown). This phenomenon can be used to isolate the target drugs from various samples using magnetic solid-phase extraction [1]. MSPE enables preconcentration of low amounts of free target compounds from a relatively large volume of the sample. As described recently, crystal violet and malachite green (both dyes being possible carcinogens) present in water in concentrations 1 ng mL^{-1} have been successfully preconcentrated from one liter of water using the same affinity adsorbent [4]. Due to the magnetic properties of the adsorbent the preconcentration process can be also performed in samples containing suspended solids.

The specific interaction between tricyclic drugs (and most probably with their metabolites with intact tricyclic structure) may enable specific preconcentration of this important group of drugs from various biological matrices and thus simplify the subsequent analysis using appropriate analytical procedures such as high performance liquid chromatography.

REFERENCES: ¹ M. Safarikova and I. Safarik (1999) *J Magn Magn Mater* **194**: 108-112. ² H. Hayatsu (1992) *J Chromatogr* **597**:37-56. ³ I. Safarik, M. Safarikova, and N. Vrchotova (1995) *Coll Czech Chem Commun* **60**: 34-42. ⁴ I. Safarik and M. Safarikova (2002) *Water Res* **196**: 196-200.

ACKNOWLEDGEMENTS: The research is a part of ILE Research Intention No. AV0Z6087904. The experimental work was supported by the NATO Linkage Grant No. LST.CLG.977500), Ministry of Education of the Czech Republic (grant project No. OC 523.80) and Grant Agency of the Czech Academy of Sciences (Project No. S6087204).