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## **ACCEPTED MANUSCRIPT**

Determination of binding properties of ampicillin in drug-human serum albumin standard solution using N-vinylpyrrolidone copolymer combined with the micellar systems

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## Abstract

It is well-known that only the unbound (free) drug fraction can achieve a pharmacological effect. Therefore the determination of free drug concentration is a very important issue in the field of pharmacology. In this study poly-1-vinyl-2-pyrrolidone (VP) crosslinked with divinylbenzene (DVB) compared with the micellar liquid chromatography (MLC) with and without pre-made drug adsorption was used for quantitative analysis of free ampicillin concentration in the standard solution of drug-human serum albumin owing to its ability to block protein adsorption.

The commonly recognized adsorption method based on drug adsorption on VP-DVB has been compared to the entirely new application of MLC with direct sample injection (DSI) not requiring pre-made adsorption. Micellar aggregates are able to solubilize various compounds therefore micellar environment can be used for direct determination of free drug concentration.

The obtained results show that the free drug concentration values obtained in the micellar systems based on cetyltrimethylammonium bromide (CTAB) (93.98  $\mu$ g L<sup>-1</sup>, 78.3%) as well as on polyoxyethylene (23) lauryl ether (Brij35) (91.15  $\mu$ g L<sup>-1</sup>, 75.9%) are similar to those obtained after the drug adsorption on VP-DVB using both RP-HPLC (95.85  $\mu$ g mL<sup>-1</sup>, 79.9%) and spectrophotometry (96.47  $\mu$ g mL<sup>-1</sup>, 80.4%). However, only %PPB (% plasma

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