**Comparative Studies of Molecularly Imprinted Polymers (MIPs) Synthesized from Different Monomers for the Purification of Lincomycin**

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**Highlights**

* Molecularly imprinted polymers (MIP) were synthesized for the purification of lincomycin (an antibiotic) in chloroform solution.
* Three monomers (methacrylic acid, trifluoromethyl acrylic acid, and acrylamide) were investigated, respectively, for their performance in related MIPs.
* Separation mechanisms, equilibrium, kinetics and operation parameters were studied. Industrial fermentation solution was used to examine the performance of the MIPs.

**1. Introduction**

With strong antibacterial effect against gram-positive bacterium and inhibitory effect to mycoplasma, lincomycin has attracted strong interest in global pharmaceutical industries [1, 2]. It possesses such advantages of: broad antimicrobial spectra, strong antibacterial ability, low toxicity and convenient administration, etc.. The industrial purification process of lincomycin involves mainly the extraction of fermentation products with butyl-alcohol or mixed alcohol[2, 3]. The crude product is obtained by crystallization process, following repeatedly extracting, concentrating and bleaching processes. A few companies utilized the hydrochloric acid stripping technique after extraction, thereafter the crystals are obtained by bleaching/crystallizing with acetone. These technologies suffered from such disadvantages as: high cost, low yield, high material and energy consumption, and environmental pollutions, etc. [3]. The fermentation solution of Lincomycin contains mainly lincomycin A, as well as a few analogues such as lincomycin B, C and D, which are known for less antibacterial activity but toxic side effects. Therefore, new technology is needed for high purity and low-cost production of the antibiotics.

Molecular imprinting is a new technology to prepare adsorbents with highly specific affinity (selectivity). The molecularly imprinted polymers (or MIPs), whose spatial structure and binding sites were designated to match with the template molecules, shed new lights for the separation of large/biomolecules out of the complicated mixture solutions[4]. The spatial structure and physicochemical properties of the template molecules are created in the MIP which can identify the template molecules effectively from a complex mixture solution [5, 6]. This technology has found numerous application in chemical[7-9], biological [10], medicine[11-13] and other fields.

**2. Methods**

Lincomycin was used as the template molecule, ethylene glycol dimethacrylate as the cross-linker. Three MIPs were synthesized, respectively, with different functional monomers (namely, methacrylic acid, trifluoromethyl acrylic acid, and acrylamide) via a two-step swelling method, and grown on the polystyrene microspheres. The related non-imprinted polymers (NIP) were also synthesized using the same procedures as MIPs, nut without adding the template molecules. The 6 samples were denoted as: MIPTF-MAA and NIPTF-MAA; MIPMAA and NIPMAA; MIPAM and NIPAM, respectively.

The MIP/NIP samples were structurally characterized using a number of instruments (SEM, FTIR, XRD, Particle size analyser, etc.), which suggested that the MIP was successfully grown on the substrates with homogeneous structure. Adsorption studies were performed in aqueous solution, chloroform solution and industrial extracts solutions, respectively. Fixed bed column breakthrough experiments were also performed to examine the kinetics and reusability of the MIPs.

**3. Results and discussion**

Figure1a shows the adsorption of lincomycin on MIPs prepared by different functional monomers. The initial concentration was fixed with a dosage of 50mg-adsorbents/20ml-chloroform solutions. We see that the adsorption capacity increases steadily with the lincomycin concentration, while the adsorption on NIPs gradually approaches to its saturation. Regardless of MIPs or NIPs, the adsorption capacity is gradually decreasing in order of TF-MAA, MAA, and AM. This is mainly attributed to the molecular structure of lincomycin. Lincomycin shows alkaline because of an amino group in molecule, so the imprinted polymer prepared by acid functional monomer has better adsorption capacity, especially the TF-MAA with three fluorine atom substituents. To MIPs and NIPs prepared by the same functional monomer, the binding capacity of MIPs is obviously higher than NIPs; this gap increases with lincomycin concentration’s increase. It indicated that the spatial structure and binding sites matched with lincomycin formed in the synthesis process, which made MIPs better adsorbents than NIPs, which are absent of the special recognition ability for lincomycin.

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| **Figure 1.** Adsorption isotherms of MIPTF-MAA (a,d) and NIPTF-MAA, (b,e)--Adsorption isotherm of MIPMAA and NIPMAA, (c,f)--Adsorption isotherm of MIPAM and NIPAM | Figure 2 The adsorption capacities of lincomycin on MIP, NIP, and activated carbon in two solutions (left: pure lincomycin solution from Lab; right: raw fermentation extracts solution from industry)  |

Figure 1b shows the comparative studies for MIP with activated carbon and the NIPs. It was found that MIP presented a moderately higher capacity in pure solution of lincomycin, but a significantly higher capacity (more than doubled) in the practical lincomycin fermentation solution extracts, confirming the superior performance (specific affinity/selectivity) of the MIP over NIP and carbon for this application.

The adsorption/desorption kinetics were also studied on MIPMA in a fixed bed column. The breakthrough curves were found to be well fitted by the Thomas model and the fixed bed has the good regeneration capability with the elution ratio > 93%.

**4. Conclusions**

With lincomycin as the template molecule, 3 different MIPs were prepared using TF-MAA, MAA and AM as functional monomers, respectively. Adsorption studies showed that MAA as the optimal functional monomer for this application. By comparing the lincomycin adsorption on MIP, NIP, as well as on activated carbon, it was found that MIPs gave better specific recognition and an adsorption capacity upto 180 μmol/g, which is higher than NIP and carbon in pure solution This difference became much higher (~ 2.5 times) for the adsorption in the practical fermentation solution extracts. Column adsorption experiment indicated that MIP had good separation effect for lincomycin. The fitting results of the kinetic model indicated that Thomas model gave a good correlation to the breakthrough curves (R2=0.98). Elution-regeneration experiments showed that the adsorption column had good desorption and regeneration capacities, with an elution ratio of 93.8 % while the breakthrough curves little changed in many cycles. These results proved that the MIP is a promising adsorbent for industrial separation and purification of lincomycin.

**References**

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