DESIGNING AND MODELING PORE SIZE AND STRUCTURAL PROPERTIES OF MESOCELLULAR SILICA FOAMS FOR ENZYME ADSORPTION

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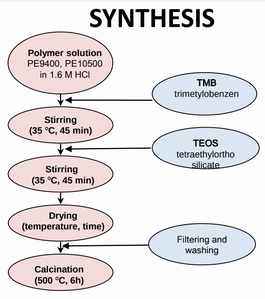
ABSTRACT

Studies of protein adsorption from solutions on the carbonaceous and mesoporous silica materials are of great importance with regard to their prominent role in bio-nanotechnology [1]. Therefore, it is important to understand mechanisms of sorption processes of the compounds showing biological activity at different interface boundaries. Mesoporous silica materials are widely utilized as carriers of active substances due to their highly developed internal structure and wider pores which are suitable for the processes involving substances with large molecular sizes. MCF adsorbents with different porosity were synthesized by using the nonionic triblock Pluronic copolymers as templates and trimethylbenzene as organic cosolvent acting as pore expanding agent. This method was used to prepare MCF materials with various structure properties according to a modified procedure described in the papers [2,3].

The structural and textural properties of the adsorbed protein layer on the mesoporous silica surface were thoroughly characterized by the UV-vis diffuse reflectance spectroscopy (UV-vis DRS) and nitrogen adsorption/desorption isotherms. Additionally, the electrochemical character and surface charge of the MCF material without and with adsorbed protein molecules were estimated by potentiometric titration. It was found that the electrochemical character of pure silica surface is clearly different than for the silica surface with adsorbed protein layer. Pure MCF adsorbents have point of zero charge, pH_{pzc}, near 4.9, however, as a result of protein adsorption, their surfaces change electrochemical properties (become amphiphilic), with pH_{pzc} near 6.5 (close to pH_{pzc} of proteins).

RESEARCH MATERIALS

SYNTHESIS



MCF
(Mesocellular foams)

- A template polymer molecule having the structure PEO-PPO-PEO (Pluronic) has an amphi-philic character.
- **MCF is a mesoporous** materials consist of a three-dimensional pore structure of uniform large spherical pore cells interconnected by narrows window pores (also described as "inkbottled)

BSA
(Bovine serum albumin)

- Molecule dimensions: 14nm × 4nm × 4nm (ellipsoidal shape) or 8 nm × 3 nm (heart shape).
- Molecular weight: 66.5kDa.
- Isoelectric point: IEP 4.7.
- BSA is a single polypeptide chain 583 constructed of corrugated amino acids.

OVA
(Ovalbumin)

- Molecule dimensions: 7nm×4.5nm×5nm (ellipsoidal shape).
- Molecular weight: 45kDa.
- Isoelectric point: IEP 4.6-4.7.
- OVA is composed of approximately 385 amino acids containing two phosphate groups per mole, and mannose and glucose residues.

STRUCTURAL PROPERTIES OF MESOPOROUS SILICAS

MEASUREMENT AND ANALYSIS OF POROUS SILICA STRUCTURE:

- The surface and structural properties of silica adsorbents were studied by the low temperature nitrogen adsorption/desorption isotherms at 77K using the ASAP2020 sorption analyzer (Accelerated Surface Area and Porosimetry, Micromeritics Instrument Corp., USA).
- Before the experiment the samples were degassed (5mmHg) at 413 K for 24h.
- The adsorption/desorption isotherms were used to analyze the structure of studied silica adsorbents: the BET specific surface area (S_{BET}), the micropore area (S_{mic}), the external surface area (S_{ext}), the total pore volume (V_t), the micropore volume (V_{mic}), the average pore diameter (D_{av}), the average hydraulic pore diameter (D_{hy}) were estimated .

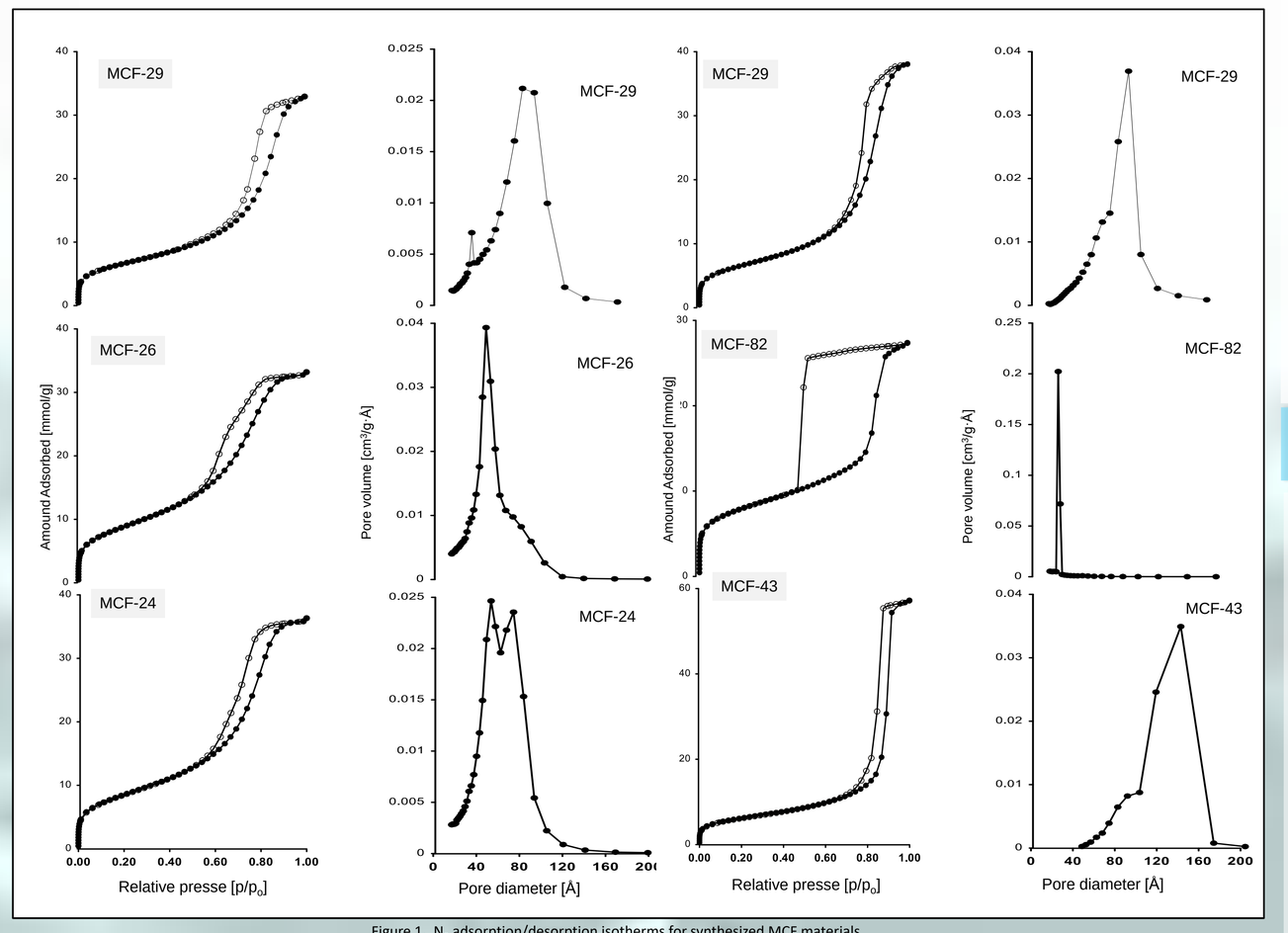


Figure 1. N_2 adsorption/desorption isotherms for synthesized MCF materials

Adsorbent	S_{BET} [m²/g]	S_{mic} [m²/g]	S_{ext} [m²/g]	V_t [cm³/g]	V_{mic} [cm³/g]	D_{av} [Å]	D_{hy} [nm]
MCF-26	716	-	724	1.15	-	52	6.42
MCF-24	685	11	674	1.26	0.002	59	7.36
MCF - 82	**630**	**224**	**406**	**0.95**	**0.098**	**27**	**6.03**
MCF-45	522	34	489	1.32	0.015	81	10.11
MCF-29	522	39	484	1.14	0.018	74	8.73
MCF-43	**486**	**52**	**434**	**1.98**	**0.026**	**126**	**16.29**

Table 1. The characteristics of mesoporous silica materials.

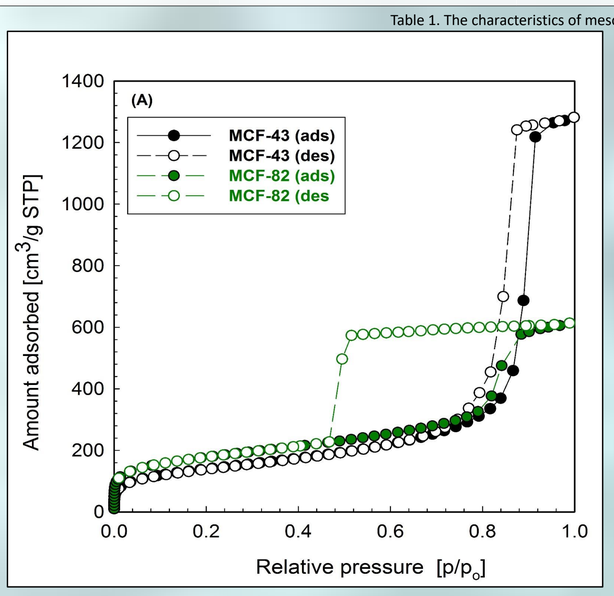


Figure 2. Comparison of N_2 adsorption/desorption isotherms for MCF-43 and MCF-82 materials.

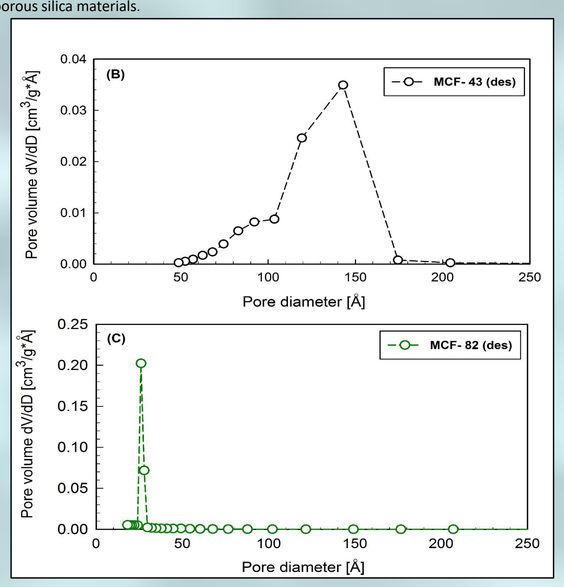


Figure 3. BJH pore size distributions calculated from desorption branch of isotherms for MCF-43 and MCF-82.

PROTEINS ADSORPTION EQUILIBRIUM

MEASUREMENTS OF ADSORPTION EQUILIBRIUM OF PROTEINS – THE METHOD:

- The adsorption isotherms were measured spectrophotometrically by using a Cary 100 UV-Vis apparatus (Varian Inc., Australia) at λ=278 nm to calculate equilibrium concentrations for proteins (BSA and OVA).
- Initial adsorbate concentrations: c_0=1,2,3,4,5 mg/ml; phosphate buffered saline with pH=7.4; adsorbent mass: 0.1 or 0.2 g.
- The protein adsorption isotherms were measured for aqueous solutions at pH=7.4 on the MCF materials by means of static method; adsorption vessels were shaken for 24 h in the incubator shaker (New Brunswick Scientific Innova 40R Model). The shaker was set up at 25°C and 110 rpm speed.
- The adsorbed amount of protein was calculated from experimental data using mass balance.

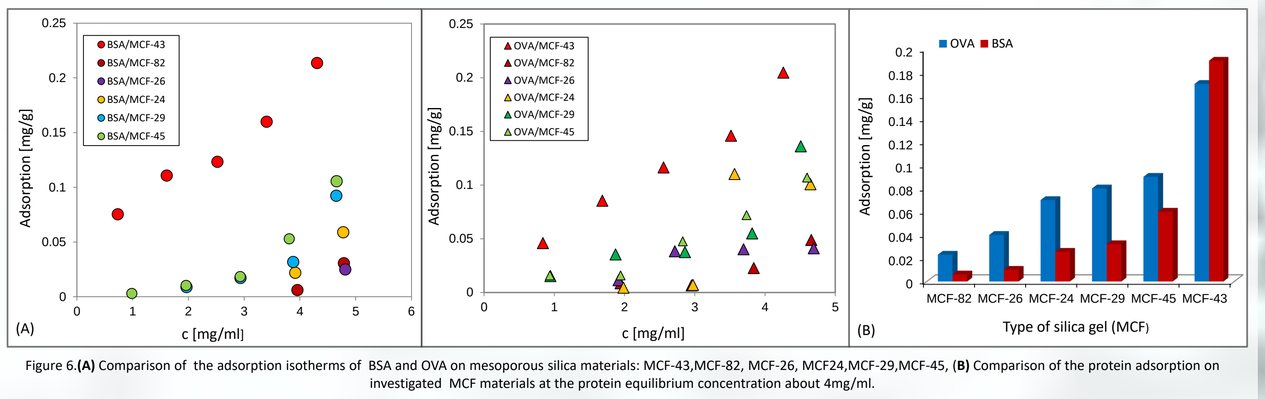


Figure 6.**(A)** Comparison of the adsorption isotherms of BSA and OVA on mesoporous silica materials: MCF-43,MCF-82, MCF-26, MCF24,MCF-29,MCF-45, **(B)** Comparison of the protein adsorption on investigated MCF materials at the protein equilibrium concentration about 4mg/ml.

PROTEINS ADSORPTION KINETICS

MEASURING METHOD OF ADSORPTION KINETICS OF THE PROTEIN :

- The adsorption kinetics measurements were carried out by using UV-Vis spectrophotometer with a quartz flow cell. Adsorption process was conducted in an external vessel, from which at definite time intervals the solution samples were collected automatically to the flow cell, the entire absorbance UV spectra in the range λ=200 - 400 nm were recorded and then the collected solution was returned to the reaction vessel.
- Initial adsorbate concentration: c_0=0.4 mg/ml; phosphate buffered saline with pH=7.4; adsorbent mass: 0.25 g.

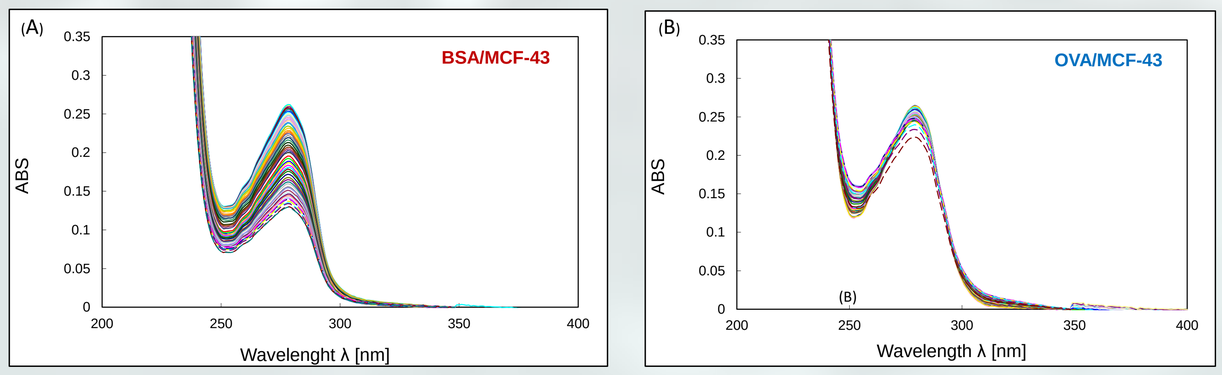


Figure 7. **(A)** Absorption spectra collected in the UV range during measurement of proteins adsorption kinetics on the surface of MCF-43 material,

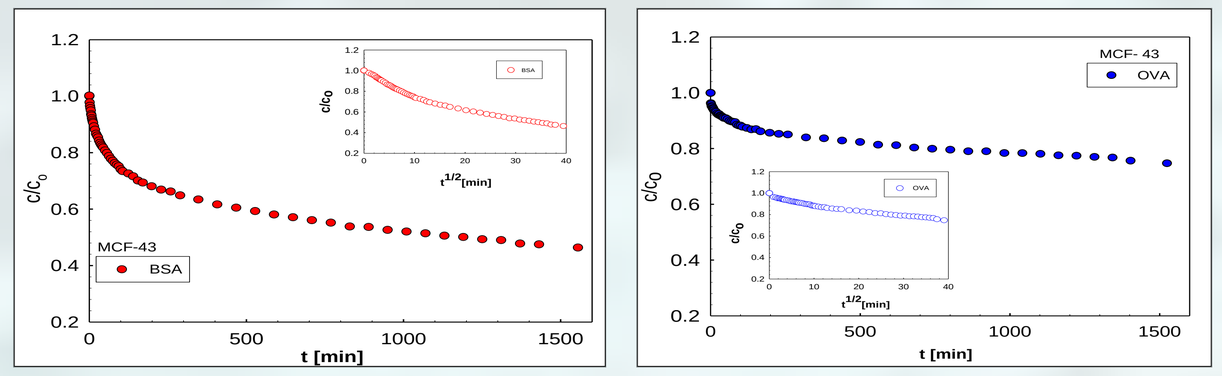


Figure 8.Kinetics of adsorption of BSA i OVA protein on the MCF-43 (relative concentration ~ time and relative concentration ~ square root of time).

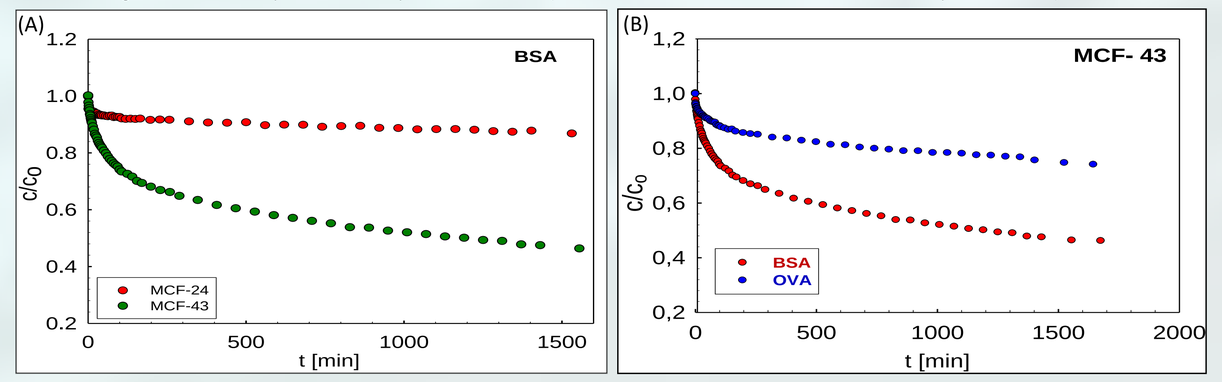


Figure 9. **(A)** Comparison of concentration profiles of BSA on the MCF-24 and MCF-43 (relative concentration ~ time); **(B)** Comparison of concentration profiles of BSA and OVA on MCF- 43 material (relative concentration ~ time).

POTENTIOMETRIC TITRATION

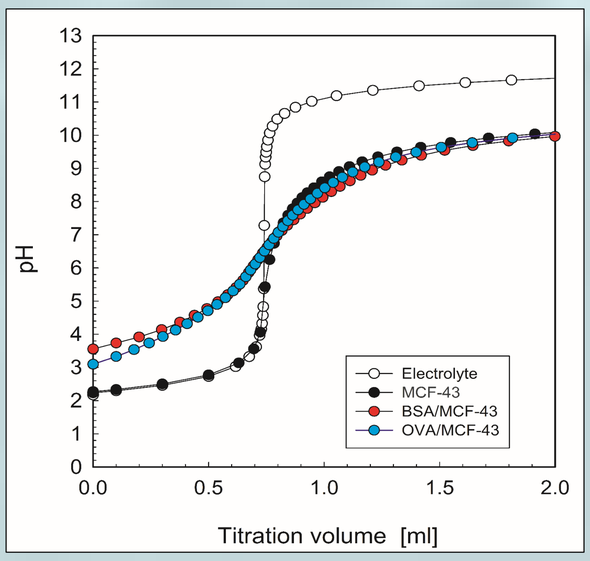


Figure 4. Potentiometric titration of following samples: pure MCF-43 silica material and MCF-43 silica having the BSA and OVA adsorption layers (i.e. BSA/MCF-43 and OVA/MCF-43, respectively).

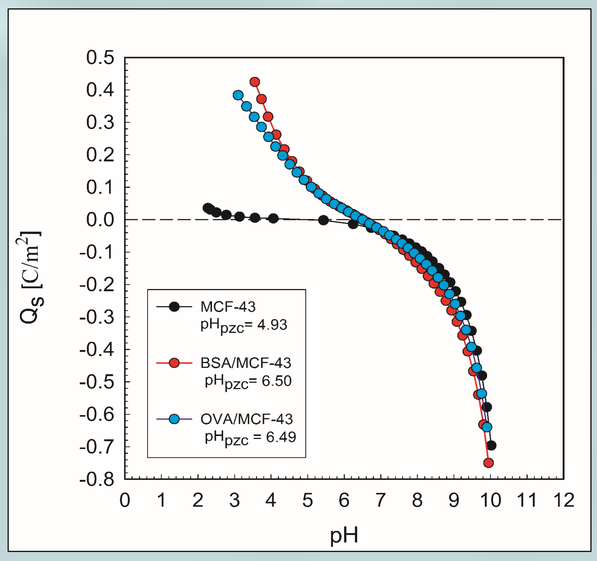
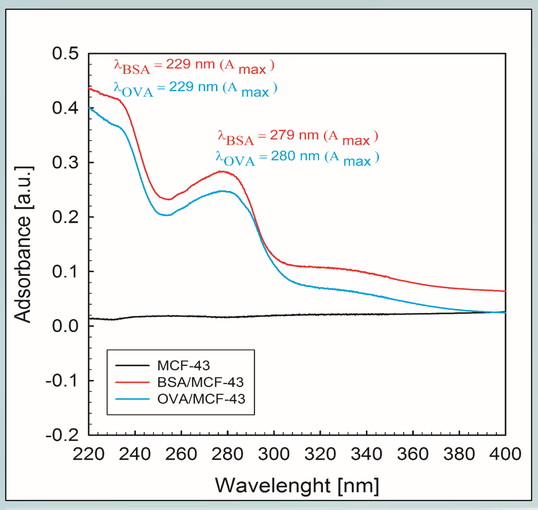


Figure 5. Dependence of surface charge density on pH for the MCF-43 and BSA/MCF-43 and OVA/MCF-43 samples. The measurements were carried out by potentiometric titration for ionic strength *I*= 0.1 mol/L.

UV-VIS DIFFUSE REFLECTANCE SPECTROSCOPY



UV-vis DRS results also shown that the MCF-43 silica surface modified by BSA and OVA adsorption molecules exhibits the well-known absorbance maximum at 279 nm and 229 nm, respectively The maximum spectrum of BSA at 279 nm is due to the π→ π* transition of the aromatic amino acids tryptophan (Trp) and tyrosine (Tyr) and to a small extent, by the absorbance of cystine (i.e. of disulfide bonds). On the other hand, the maximum spectrum at 229 nm is attributed to the n→ π* transition of the carboxylic group in peptide bond

Figure 10. Diffuse reflectance UV-vis spectra of MCF-43 material with adsorbed protein layers. Adsorption conditions: c_0 = 5 mg/ml (initial concentration of proteins solution); t_{ads} = 24 h (time available for adsorption); T = 298 K (temperature process adsorption); pH = 7.4 (phosphate buffered saline).

CONCLUSIONS

- The porosity (pore size, pore volume, specific surface area and a grain size) silica material have a great influence on the rate of adsorption process.
- Adsorption process of proteins on microporous silica adsorbents is slow and limited by diffusion of molecules into internal adsorbent porous structure.
- Adsorption of protein molecules occurs most efficiently in the case of MCF materials having larger pore sizes.
- Adsorption layer of the BSA molecules on the MCF-43 materials is thicker (due to different structures of the MCF materials and protein molecules).
- The fastest adsorption of both albumins on MCF-43 material occurs in the initial stage of the adsorption process (easier access to the unfilled pores of the adsorbent).
- There is a correlation between the size of the protein molecule and the adsorption kinetic.
- For larger molecules of protein (BSA) the total rate of adsorption process on the MCF-43 adsorbent is greater than in the case of OVA adsorption.
- DRS UV-vis spectroscopy showed existence of protein molecules on the MCF surface.
- Potentiometric titration results before and after the protein adsorption on MCF material showed different electrochemical character of surface.

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