

Synthesis and determination of dissociation equilibrium constants of novel metal chelating agent against Alzheimer's disease

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Abstract. In this paper, we have synthesized a new metal chelating agent HPM and characterized it by NMR, IR and LC-MS/MS measurements. The HPM obtained three pKa values: pKa₁=3.1988, pKa₂=4.4157 and pKa₃=9.0091. The pKa₁ is due to the carboxyl group on the C11 position, the pKa₂ is due to the hydroxyl group on the C6 position and the pKa₃ is due to the NH²⁺ on the N8 position.

Keywords: Alzheimer's disease, metal chelating agent, dissociation equilibrium constant.

1. Introduction

Alzheimer's disease (AD) is a degenerative neurological disease characterized by synaptic loss, cognitive decline and progressive neuronal death. The main clinical manifestations are cognitive decline and behavioral Barriers^[1]. According to the World Alzheimer's Disease 2021 Report, more than 55 million people worldwide suffer from AD, and is expected to reach 78 million by 2030^[2]. However, some studies have shown that the occurrence of the disease is related to the metabolic disorder of metal ions, and may be related to the over expression of aluminum ions and iron ions. We have a strong interest in the discovery of novel metal chelating agents to regulate and control the content of aluminum and iron ions in the body.

The aim of the paper is to design and synthesize a new metal chelating agent HPM, calculate the dissociation equilibrium constants and physical distribution of HPM by potentiometric titrations. This work can provide the foundation for the chelation formation and distribution of ions. It provides a new idea for the prevention and treatment of AD to guarantee the human life and health.

2. Experiment

Materials: All chemicals were commercially available and of pure grade. Methanol, potassium hydroxide, sodium hydroxide, sodium borohydride, concentrated hydrochloric acid, ether and β-alanine were purchased by Macklin and 4-hydroxynicotinic aldehyde (98.42%) by Leyan.

Apparatus: The ¹H NMR and ¹³C NMR were made with BRUKER AVANCE 400, using deuterium dimethyl sulfoxide (DMSO) as solvent and tetramethylsilane (TMS) as calibration. IR spectra was made with Nicolet IS10. MS spectra was made with LC-MS/MS by Thermo Fisher TSQ Fortis. The potentiometric titrations made with automatic potentiometric titrator by Beijing Xianquweifeng ZDJ-3DX.

The synthesis of HPM: The synthesis of the HPM has been performed referencing a procedure reported in the literature^[3], starting from 4-hydroxynicotinic aldehyde (A) through the step a and b reported in Figure 1.

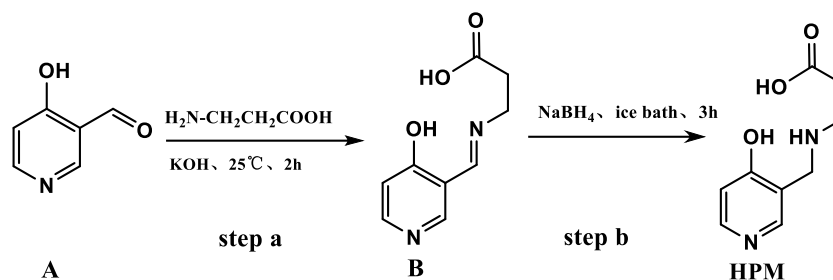


Fig. 1 The synthesis of HPM

In step a, β -alanine (1 mmol, 94.38mg) and KOH (1 mmol, 67.23mg) were added with 10mL of MeOH in a 100ml flask at 50°C water base, equipped with condenser and magnetic stirrer. The colorless clean solution was quickly heated and refluxed for 1h. After colling, the 4-hydroxynicotinic aldehyde (1mmol, 124.31mg) A was added drop wise with 25ml of MeOH. Stir the mixture for 2h at room temperature to form the intermediate Schiff base B. During the reaction, the color of the solution gradually deepened, TLC monitored the completion of the reaction. Stop the reaction and cool mixture down.

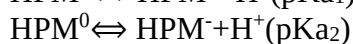
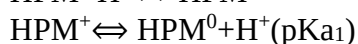
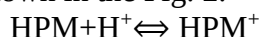
In step b, NaBH_4 (2mmol, 58.91mg) in 20ml MeOH containing three drops of 2mol/L NaOH solution was added drop wise into the yellow solution in 0.5h. The solution was stirred for 3h in an ice bath. Acidify the solution with concentrated HCl to a pH of 5.0-6.0, evaporate the solution entirely and extract the solution with dry MeOH, filter off the resulting solid. And then evaporate the solution again, using dry MeOH firstly to wash it and then Et_2O was added to remove the impurities with low polarity. Finally dry the solid in vacuum drying chamber at 50°C for 24h.

Yield: 121.87mg (62.18%); mp: $220-221^\circ\text{C}$. ^1H NMR (400 MHz, DMSO) : δ 8.02 (s, 1H), 7.78 (d, $J = 7.2$ Hz, 1H), 6.23 (d, $J = 7.2$ Hz, 1H), 3.90 (s, 2H), 3.02 (t, $J = 7.1$ Hz, 2H), 2.74 (t, $J = 7.1$ Hz, 2H). ^{13}C NMR (101 MHz, DMSO) : δ 177.46 (s), 172.05 (s), 138.97 (s), 138.38 (s), 119.07 (s), 116.18 (s), 44.11 (s), 42.00 (s), 30.26 (s). IR (KBr, cm^{-1}): $\nu(\text{OH})$ 3416, $\nu(\text{NH})$ 3091, $\nu(\text{COO}^-)$ 1610, 1397. LC-MS/MS: $m/z=195.0$ ($\text{C}_9\text{H}_{11}\text{N}_2\text{O}_3^-$), $m/z=88.03$ ($\text{C}_3\text{H}_6\text{NO}_2^-$), $m/z=123.10$ ($\text{C}_6\text{H}_7\text{N}_2\text{O}^-$).

Solution equilibria: All analyte concentrations were expressed in the molality scale (mol/kg of water, m). For potentiometric measurement, working solutions of HCl (0.1M), NaOH (0.1M), HPM (10mM) were prepared and standardized. The starting solution for each titration and all working solutions contained known amounts of NaCl to assure a constant (Na)Cl 0.6M ionic strength during the titrations^[4,5]. The potentiometric titrations is under the water base at $25 \pm 0.1^\circ\text{C}$ and nitrogen condition. All dissociation equilibrium constants (pK_a) were calculated using the computer program PITMAP^[6].

3. Results and discussion

We obtained three pK_a values from our potentiometric titrations: $\text{pK}_{a1}=3.1988$, $\text{pK}_{a2}=4.4157$, $\text{pK}_{a3}=9.0091$. The pK_{a1} is due to the carboxyl group on the C11 position, the pK_{a2} is due to the hydroxyl group on the C6 position and the pK_{a3} is due to the NH^{2+} on the N8 position. At the same time, we also found that there may be a fourth pK_a , N on pyridine, because the N on pyridine is not involved in the subsequent chelation process, so we don't consider the effect of N on pyridine on the experimental results. At the same time, the different experimental environments, sample purity and fitting methods will cause the different experimental results. The dissociation process of HPM is shown in the Fig. 2.



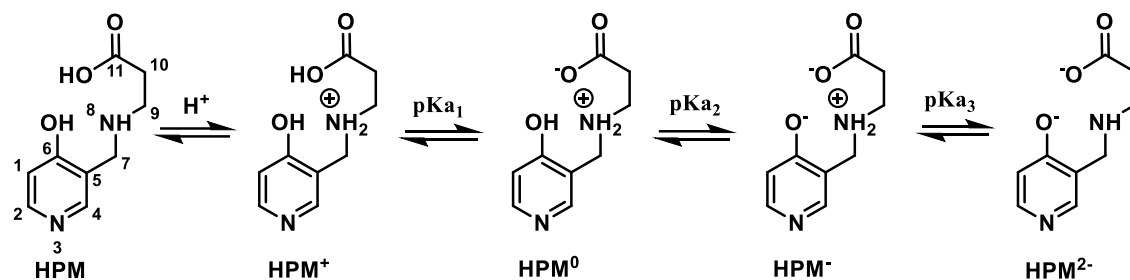


Fig. 2 The dissociation process of HPM

We discussed the dissociation equilibrium process of HPM. The PITMAP can not only fit the potentiometric titration data to calculate pK_a , but also calculate the morphological distribution of substances. The fitted pH values were brought into the PITMAP, and the calculation range of pH refers to the range of pH in the titration experiment, which is $pH=3.00-10.00$. There are 101 points in the selected range, the X axis is made with 0.07 pH as the interval, the Y axis is made with the percentage of species of HPM (%). The physical distribution of HPM in aqueous solution under different pH conditions is shown in Fig. 3.

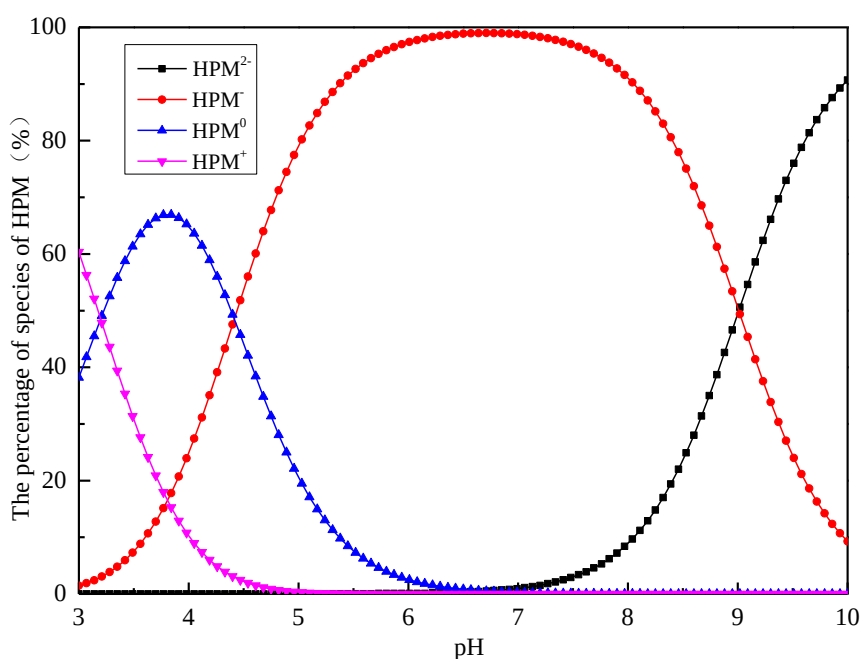


Fig. 3 The physical distribution of HPM in aqueous solution

From the analysis of Fig. 3, when $pH=3.00-5.73$, HPM mainly exists three forms in aqueous solution: HPM^+ , HPM^0 and HPM^- ; when $pH=5.73-6.01$, HPM mainly exists four forms in aqueous solution: HPM^+ , HPM^0 , HPM^- and HPM^{2-} , at this stage, HPM^+ is almost exhausted, but HPM^{2-} is just formed; when $pH=6.01-8.60$, HPM mainly exists three forms in aqueous solution: HPM^0 , HPM^- and HPM^{2-} . When $pH=8.60-10.00$, HPM mainly exists two forms in aqueous solution: HPM^- and HPM^{2-} .

4. Conclusions

We have designed and synthesized a new metal chelating agent HPM and characterized it by NMR, IR and LC-MS/MS measurements. We have calculated the pK_a values of HPM by the data of potentiometric titrations, using the PITMAP. The three pK_a values of HPM were $pK_{a1}=3.1988$, $pK_{a2}=4.4157$ and $pK_{a3}=9.0091$. We also shown the physical distribution of HPM in this paper.

Acknowledgments

This work was financially supported by the National Natural Science Foundation of China (2186010116).

References

- [1] Jinxiu Wang, Gao Fan, Research progress on the mechanism of A β affecting Alzheimer's disease, Life Science Research, 2021,25(01):53-57.
- [2] World Alzheimer's Report, Alzheimer's disease International, 2021.
- [3] Chang Tong Yang, Boujemaa Moubarki, Interconversion of a Monomer and Two Coordination Polymers of a Copper (II)-Reduced Schiff Base Ligand-1,10-Phenanthroline Complex Based on Hydrogen- and Coordinative-Bonding, Inorg. Chem. 2001(40)5934-5941.
- [4] G. Giorgio Bombi, Valerio B. Di Marco, 2-Hydroxy-3-carboxy-dihydrocinnamic acid: Complexation properties towards aluminium (III) and iron (III), Polyhedron, 26(2007) 3419-3427.
- [5] G. Giorgio Bombi, Rehemani Aikebaier, Complexation of 2,6-pyridinedicarboxylic and 2,6-pyridinediacetic acids towards aluminium (III) and iron (III), Polyhedron, 28(2009) 327-335.
- [6] V. B. Di Marco, Ph.D. Thesis, University of Padova, 1998.