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An Optimized Synthesis of Manganese *meso*-Tetra(4-sulfonatophenyl)porphine: A Tumor-Selective MRI Contrast Agent

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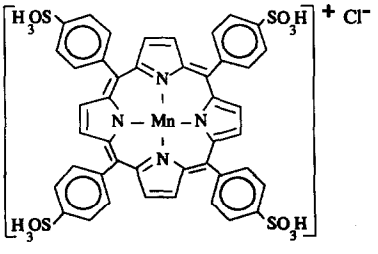
Abstract: The preparation of the potential magnetic resonance imaging (MRI) tumor-selective contrast agent manganese meso-tetra(4-sulfonatophenyl)porphine ($MnTPPS_4$) was optimized to yield a product with high purity (≥98%) in large amounts (> 1 g). The presented simplified purification method may contribute to the application of $MnTPPS_4$ in clinical trials.

INTRODUCTION

In the medical field, porphyrins have become of growing interest in both, diagnosis and therapy of cancers:[1-6] The selective accumulation of these pigments in tumor[7-11] has been used for tumor diagnosis by magnetic resonance imaging.[1,12-17] For photodynamic therapy of tumors[3,18] the behavior of the porphyrins as light traps has been used. Synthetic metal porphyrins such as meso-tetra(4-sulfonatophenyl)porphine ($MnTPPS_4$, **1**) are of particular interest, for in the cerebrum - in contrast to conventional contrast agents - they exclusively enhance neoplastic, but not edematous or normal tissue.[19,20] However, $MnTPPS_4$ is - for its sulfonated phenyl groups - a very polar porphyrin. Therefore, commercially available $MnTPPS_4$ is usually contaminated with unidentified by-products in considerable amounts.[21] Such contamination is, however, not acceptable in medical field, where unknown substances can lead to severe negative reactions. To overcome this problem, we optimized the synthesis and purification procedure of this important potential NMR contrast agent, $MnTPPS_4$.



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DISCUSSION

Lyon et al.[21] reported for the $MnTPPS_4$ (Midcentury, Posen, Illinois, USA), used in their studies, a purity of about 60%. They assumed, that the other 40% of this substance consisted of acetic acid (≈20%) and water (≈20%). Porphyrin-selling companies[22] state, that their products contain considerable amounts of water and solvents in variable quantities. They advise customers to check the true porphyrin content of their products spectrophotometrically before using it. The identities of these compounds, which are included in the industrial product $MnTPPS_4$, usually remain unknown. Therefore, it can not be excluded, that these by-products may have influence on subjects under investigation like toxicity, NMR relaxation, contrast-enhancement etc. For safe investigations and the determination of reproducible data, however, it is essential to analyze quality and quantity of these compounds or, preferentially, to exclude the presence of unknown compounds. High purity (in even large quantities) as required for such in vivo investigations can be obtained by the procedure developed in our laboratories.

CONCLUSION

$MnTPPS_4$ of the excellent quality obtained by our method will produce reliable results in quantitative investigations. With the exclusion of by-products the volume of solvents necessary for applications of contrast agents will be minimized. Furthermore, toxic effects of unidentified by-products can be excluded, when the purity of the product to be applied is close to 100%.

EXPERIMENTAL

$MnTPPS_4$ was synthesized from *meso*-tetra(4-sulfonatophenyl)porphine (H_2TPPS_4) and manganese(II)-acetate. H_2TPPS was either synthesized by described methods[23-25] or purchased.[22] H_2TPPS_4 and $Mn^{2+}(CH_3COO^-)_2 \times 4\ H_2O$ (Fluka, Buchs, Switzerland) were not purified for the synthesis of the metal complex.

Manganese *meso*-tetra(4-sulfonato-phenyl)porphine

1g H_2TPPS_4 (1 mmol) and 3 g $Mn^{2+}(CH_3COO^-)_2 \times 4\ H_2O$ (12 mmol) were heated in 100 ml acetic acid (100%, Fluka, Buchs, Switzerland) under reflux (115°C) for 8h. Air was bubbled through the refluxing solution by a suction pump to stabilize the complex by oxidation of manganese(II).[26] After 8h the absorption spectrum of the crude product in aqueous solution (350-700 nm, Figure 1) was recorded (Shimadzu UV 2100 spectrophotometer, Shimadzu Corporation, Kyoto, Japan). The completion of the reaction was indicated by the disappearence of the Soret band of H_2TPPS at 410 nm (full line, Figure 2). Additionally the presence of unmetallated H_2TPPS was controlled by its intensive red fluorescence at 630 nm, i.e. after irradation with a Hg-lamp at 350 nm. Figure 2 presents absorption spectra (350-500 nm) of H_2TPPS (full line) and MnTPPS (dotted line) in equal concentration to demonstrate the clear difference of both spectra. The hot solution of $Mn^{2+}(CH_3COO^-)_2$ in acetic acid was decanted and the remaining crude product was washed at least three times with 50 ml hot acetic acid (80°C) to remove excess $Mn^{2+}(CH_3COO^-)_2$. Afterwards, the crude product was washed at least three times with 50 ml portions of hot ethanol (70°C) to remove acetic acid. The remaining crude product was dissolved in an aqueous solution of Na_2CO_3 in 200 ml water (pH≈10) and passed through a qualitative filter paper (595 ½ Schleicher & Schuell, Dassel, Germany). The filtered solution was acidified (pH < 1) with concentrated hydrochloric acid (37%, Merck, Darmstadt, Germany), leading to the occurrence of a golden colored colloid. After storage in a refrigerator (4°C) overnight the solution was centrifugated (3000 rpm, 1h), the supernatant decanted and the remaining product dried overnight in an oven (100°C). Then the substance

was solved again in an aqueous solution of Na_2CO_3 (pH≈10), filtered, acidified, centrifugated and dried in the above described manner. The resulting product was further dried over KOH in a desiccator (vacuum). The final product (1.0 g, 0.8 mmol, 80% yield) with a dark green, slightly metallic color had a purity of better than 98%, determined optically with an absorption coefficient ε_{467} of 97.6 $l \cdot mmol^{-1} \cdot cm^{-1}$.[21] The results of elemental analysis (Mikroanalytisches Labor Pascher, Remagen, Germany) were in excellent agreement with the theoretical values of the structure formula $MnTPPS_4^+Cl^-$: Anal. Calcd. for $C_{44}H_{28}N_4MnO_{12}S_4Cl$: C, 51.6; N, 5.5; O, 18.8; Mn, 5.4; S, 12.5; H, 2.7; Cl, 3.5%. Found: C, 51.6; N, 5.5; O, 21.3; Mn, 5.5; S, 12.2; H, 3.0; Cl, 1.1%. The larger amount of oxygen and hydrogen and the smaller amount of chlorine found in the synthesized MnTPPS may be a result of hydroxyl groups, replacing 2/3 of the chlorine. This replacement has probably occurred during the drying of $MnTPPS_4$ over KOH in vaccuo.

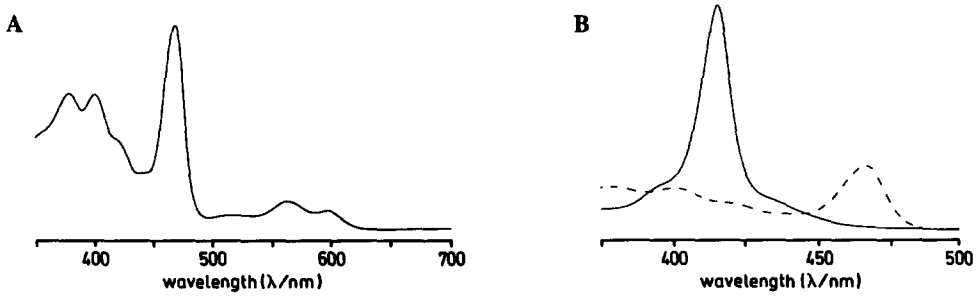

Fig. 1. A: Optical absorption spectrum of $MnTPPS_4$ in aqueous solution. The shoulder at 410 nm indicates the exclusive presence of the metallated porphine. B: Absorption spectrum of equimolar aqueous solutions of H_2TPPS_4 (full line) and $MnTPPS_4$ (dotted line). The dominating Soret band of H_2TPPS_4 at 410 nm ($\varepsilon \approx 500$ $l \cdot mol^{-1} \cdot cm^{-1}$) totally overlaps the $MnTPPS_4$ spectrum in this bandwidth. Therefore the absence of a peak at 410 nm (conf. Fig.1) clearly indicates the exclusive presence of $MnTPPS_4$.

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