学校编码：10384　　分类号_____密级_____

学号：20720060153281　　UDC_____

厦门大学

博　士　学　位　论　文

N-硬脂酸-O-羧甲基壳聚糖衍生物自组装纳米载药系统的研究

Study of self-aggregated nanoparticles of N-stearic acid -O-carboxylmethyl chitosan derivatives and the primary application as the novel carriers of drugs

罗芳洪

指导教师姓名：　张其清　教授

专　业　名　称：　高分子化学与物理

论文提交日期：　2012 年 12 月

论文答辩日期：　2013 年 01 月

学位授予日期：　2013 年　月

答辩委员会主席：_____

评　阅　人：_____

2013 年 1 月

厦门大学学位论文原创性声明

本人呈交的学位论文是本人在导师指导下,独立完成的研究成果。本人在论文写作中参考其他个人或集体已经发表的研究成果，均在文中以适当方式明确标明，并符合法律规范和《厦门大学研究生学术活动规范（试行）》。

另外,该学位论文为(　　)课题(组)的研究成果，获得（　　）课题（组）经费或实验室的资助，在（　　）实验室完成。（请在以上括号内填写课题或课题组负责人或实验室名称，未有此项声明内容的，可以不作特别声明。）

声明人（签名）：

年　　月　　日

厦门大学学位论文著作权使用声明

本人同意厦门大学根据《中华人民共和国学位条例暂行实施办法》等规定保留和使用此学位论文，并向主管部门或其指定机构送交学位论文（包括纸质版和电子版），允许学位论文进入厦门大学图书馆及其数据库被查阅、借阅。本人同意厦门大学将学位论文加入全国博士、硕士学位论文共建单位数据库进行检索，将学位论文的标题和摘要汇编出版，采用影印、缩印或者其它方式合理复制学位论文。

本学位论文属于：

（　　）1.经厦门大学保密委员会审查核定的保密学位论文，于　　年　　月　　日解密，解密后适用上述授权。

（　　）2.不保密，适用上述授权。

（请在以上相应括号内打“√”或填上相应内容。保密学位论文应是已经厦门大学保密委员会审定过的学位论文，未经厦门大学保密委员会审定的学位论文均为公开学位论文。此声明栏不填写的，默认为公开学位论文，均适用上述授权。）

声明人（签名）：

年　　月　　日

中文摘要

两亲性聚合物在水性介质中能通过自组装方式形成纳米胶束，该类胶束具有独特的核-壳结构及体内长循环和主体稳定等特点；且由于具有增强渗透滞留效应（Enhanced Permeability and Retention Effect，EPR），该类聚合物胶束能在肿瘤病变部位富集，实现对肿瘤组织的“被动靶向”作用，将其作为抗肿瘤药物载体，是提高药物疗效，降低药物毒副作用的有效策略之一。O-羧甲基壳聚糖（O-carboxyl methyl chitosan，CMC）是一种水溶性壳聚糖衍生物，保留了壳聚糖生物相容性好、可生物降解、无免疫原性、无毒性等独特优点，将其疏水改性后形成两亲性壳聚糖，具有两亲性质，在一定条件下能自组装形成纳米胶束，可作为包载疏水性药物的理想纳米药物载体。

本文从O-CMC氨基端引入硬脂酸（stearic acid，SA）合成SA-CMC聚合物，考察其在水性介质中的两亲性及临界聚集浓度（critical aggregation concentration，CAC），以紫杉醇（paclitaxel，PTX）为模型药物考察其对疏水药物的包载和释放行为；观察PTX载药纳米粒（PTX -SA-CMC NPs）对肿瘤的靶向性及体内外抗肿瘤效应，初步观察其毒性，综合评价其作为抗肿瘤靶向药物载体的可行性。主要研究成果如下：

1. 以 EDC 为偶联剂，将 SA 接枝到 O-CMC 的氨基上，一步法合成了 SA-CMC 的共聚物；采用红外光谱（FTIR）、氢核磁共振（^{1}HNMR）等手段对产物的结构进行表征。通过控制 SA 和 CMC 的投料比，合成三种不同取代度（degree of substitution，DS）的聚合物。三硝基苯磺酸(2,4,6-trinitribenzenesulfonic acid, TNBS)法检测结果：三种聚合物的 SA 取代度分别为 7.3%、13.8%和 19.5%。
2. 芘荧光探针法检测结果表明，SA-CMC 衍生物具有两亲性，在水介质中能自组装形成纳米胶束，三种聚合物的 CAC 分别为 0.0212、0.0117 和 0.0098mg/mL。探头超声法制备了 SA-CMC 自聚集纳米胶束（SA-CMC NPs）。透射电镜（TEM）观察结果表明，SA-CMC NPs 呈球状，形态规则，平均粒径和 CAC 均随着 DS 的增加而降低；SA-CMC NPs 的 Zeta-电位为负值，表明带负电荷的羧甲基位于纳米胶束表面，Zeta-电位的绝对值随着 SA 的 DS

增加而降低。自聚集的 SA-CMC NPs 为“多核模型”结构。

3. 超声透析法制备了载 PTX 的自聚集纳米胶束（PTX-SA-CMC NPs），用 HPLC 法检测其载药量（loading content，LC）和包封率（encapsulation efficiency，EE）分别可达 18.6%和 86.2%；随着药物配比的增加，PTX-SA-CMC NPs 的 LC 增加，EE 降低，粒径变大。TEM 下 PTX-SA-CMC NPs 呈规则球形，平均粒径在 106 到 123 nm 之间；PTX 从 PTX-SA-CMC NPs 的释放呈两相模式，且随着 LC 的增加，PTX 的释放速度减慢，随着释放介质 pH 值的降低，PTX 释放的速度增快，72h 的累计释放量在 73%-90%之间。
4. 制备 Cy5.5 标记的载药纳米胶束（Cy5.5-PTX-SA-CMC NPs），尾静脉注射到荷瘤裸鼠体内后，采用活体荧光成像技术追踪其在体内的组织分布。结果表明，Cy5.5-PTX-SA-CMC NPs 在荷瘤裸鼠体内具有明显的肿瘤靶向性，给药后 8h 肿瘤部位浓度最高；72h 时肿瘤部位的荧光强度为肾脏、肝脏和肺脏的 5 倍，脾脏的 8.5 倍，心脏的 34.2 倍。激光共聚焦显微镜观察显示，BEL-7402 细胞以内吞的方式实现对 Cy5.5-PTX-SA-CMC NPs 的摄取。
5. 四甲基氮唑蓝比色(MTT)法研究显示，PTX-SA-CMC NPs、Cremopher EL-PTX 和 Cremopher EL 均对细胞生长有显著的抑制作用，抑制效应为 Cremopher EL-PTX＞PTX-SA-CMC NPs＞Cremopher EL，而 SA-CMC NPs 对 BEL-7402 无明显的细胞毒性。而体内抑瘤实验结果显示，PTX-SA-CMC NPs 组的抑瘤率较 Cremopher EL-PTX 组明显提高，荷瘤鼠的生存期也明显延长。安全评价实验结果表明，PTX-SA-CMC NPs 的血液相容性好，对小鼠无明显的毒副作用，安全有效。因此，SA-CMC NPs 是一种理想的肿瘤靶向药物载体。

关键词：硬脂酸-羧甲基壳聚糖；自聚集纳米粒；紫杉醇；肿瘤靶向；药物载体。

Abstact

Amphiphilic conjugate have received increasing attention because they can form self-aggregated nanoparticles. In the aqueous phase, the hydrophobic cores of polymer ric nanoparticles are surrounded by hydrophilic outer shells. Thus, the inner core can serve as a nano-container for hydrophobic drugs. The nanoparticles can accumulate and extravasate within tumor tissue due to the prolonged circulation and enhanced permeation and retention (EPR) effect. Therefore, drug delivery using polymeric nanoparticles is an effective strategy for passive tumor targeting. The O-carboxylmethyl chitosan (O-CMC) has many good properties, such as biocompatibility, biodegradability, non-toxicity and bioadhesivity that make it suitable for use as a hydrophobic anticancer drug carrier.

This dissertation designed a series of stearic acid-modified O-carboxylmethyl chitosan (SA-CMC) conjugates and assess their potentials as drug delivery systems. The main investigations of this dissertation are shown as follows.

1）A series of SA-CMC conjugates with different degrees of substitution(DS) of the SA were synthesized by the amide linkages in the presence of EDC. The chemical structure of SA-CMC conjugates was characterized by fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (^{1}H NMR). The DS of SA-CMCs detected by 2,4,6-trinitribenzenesulfonic acid (TNBS) method were 7.3,13.8 and 19.5%, respectively.

2）The SA-CMCs were amphiphilic in nature and their self-aggregation behavior in aqueous media was evaluated by the fluorescence probe technique.The SA-CMC conjugates could form self-aggregate nanoparticles by probe sonication with the CAC of 0.0212、0.0117 and 0.0098mg/mL respectively. The mean diameters decreased as the DS of SA-CMC conjugates increased. The SA-CMC NPs were analyzed by dynamic laser light-scattering (DLLS) and transmission electron microscopy (TEM) technologies. These SA-CMC NPs were almost spherical in shape and showed

multi-core characteristic. The zeta potentials of SA-CMC NPs were negative, and the absolute values slightly decreased with DS of the SA-CMC increasing.

3）Paxtical (PTX)，as a model anticancer drug，was physically entrapped inside the SA-CMC NPs. PTX-loaded SA-CMC-2 NPs were almost spherical in shape and their size increased from 106 to 123 nm with the PTX-loading content(LC) increasing from 8.6% to 18.8%. The release behavior of PTX from SA-CMC NPs was studied in vitro by a dialysis method and the results showed that PTX was released from nanoparticles in a biphasic way.The release rate was in range of 73%-90% within 72h, which decreased with the pH of the release media increasing. This release property is advantageous to controlled and sustained drug release.

4) Cy5.5-labeled PTX-SA-CMC NPs were intravenously injected via the tail vein into BEL-7402 tumor-bearing nude mice. After injection, the time-dependant *in vivo* biodistribution and tumor targetability of Cy5.5-SA-CMC-PTX NPs were non-invasively imaged using the Explore Optix System. The results showed that Cy5.5-SA-CMC-PTX NPs present an excellent tumor targeting ability in tumor-bearing mice. The total photon counts of Cy5.5-SA-CMC-PTX NPs in tumor tissue are 34.2-fold higher than that in heart at 72h. Confocal laser scanning microscopy (CLSM) demonstrated that cellular uptake of PTX-SA-CMC NPs was in an endocytosis manner.

5) Cytotoxicity of blank SA-CMC NPs and PTX-SA-CMC NPs against BEL-7402 cells was evaluated by MTT assay. These results indicated that SA-CMC nanoparticles have no cytotoxicity against BEL-7402 cells. PTX-SA-CMC NPs showed a lower cytotoxicity against BEL-7402 cells than Cremopher EL-PTX. However, compared with Cremopher EL-PTX, PTX-SA-CMC NPs exhibited enhanced antitumor effect and less side-effect in H22-bearing mice. Therefore, these results indicated that SA-CMC NPs could be a potential carrier for the drug delivery.

Key words: Stearic acid-O-carboxymethyl chitosan derivative; Self-aggregated nanoparticle; Paxtical; Tumor targeting; Drug carrier.

目 录

Contents