

聚乙二醇-*b*-聚(D,L-丙交酯-*co*-碳酸丙二酯) 的胶束化及其药物控释研究*

高界铭¹ 郭颖志² 顾忠伟^{3**} 张兴栋³

(¹西南交通大学材料科学与工程学院 成都 610031) (²国家人口计生委科学技术研究所 北京 100081)

(³四川大学国家生物医学材料工程技术研究中心 成都 610064)

摘 要 通过开环共聚合成了由 D,L-丙交酯、碳酸丙二酯和聚乙二醇构成的两亲性嵌段共聚物(PETLA),研究了 PETLA 胶束化及药物控释行为.嵌段共聚物和胶束通过核磁共振(¹H-NMR)、荧光分光光度计、凝胶渗透色谱(GPC)、动态光散射(DLS)、透射电镜(TEM)和紫外光谱(UV)表征.实验结果发现临界胶束浓度随共聚物疏水链段长度增加而减小,胶束直径随疏水链段长度增加而增大.透射电镜照片表明载药胶束 MT1 直径为 30 ~ 40 nm,呈规则球形.体外释药表明 9-NC 以可控方式释放,突释后药物释放速率接近零级恒速.

关键词 生物降解, 聚乙二醇-*b*-聚(D,L-丙交酯-*co*-碳酸丙二酯), 胶束, 药物释放

两亲性嵌段共聚物胶束处于介观尺寸范围,具有相当窄的尺寸分布和独特的体内生理特性,用作抗肿瘤药物载体可实现药物的主/被动靶向、缓控释放和减轻药物对正常组织的毒副作用,因此受到广泛关注^[1,2].目前以聚乙二醇-聚乳酸嵌段共聚物胶束作为药物载体的研究已有大量文献报道^[3,4],但此类胶束疏水性链段的降解产物为酸性,会引起体内局部微环境的 pH 值变化,容易导致炎症反应^[5].聚碳酸酯是另一类重要的聚酯材料,具有良好的生物相容性和表面溶蚀性,其降解产物为二氧化碳和二元醇(酚).聚碳酸酯的降解产物在体内引起的 pH 值变化小,不会引发炎症反应^[6],但缓慢的降解速率是影响其广泛应用的一个重要原因^[7].此外,研究表明聚乙二醇-聚碳酸酯共聚物载药胶束难以实现药物的零级恒速释放^[8,9].若将聚乳酸和聚碳酸酯的共聚物作为两亲性嵌段共聚物的疏水链段,不仅能够有效缓解降解产物导致的 pH 值降低,还能够通过改变共聚物的组成,调节药物的释放行为.

本文通过开环共聚,合成了聚乙二醇-*b*-聚(D,L-丙交酯-*co*-碳酸丙二酯)(PETLA)两亲性嵌段共聚物,对嵌段共聚物的结构、组成以及胶束化行为进行了研究,并以 9-硝基喜树碱(9-NC)为模型药物,考察了载药胶束的体外药物释放性能.

1 实验原料

碳酸丙二酯(TMC)参照文献[10]的方法合成,粗产品用四氢呋喃/乙醚(体积比 2:1)回流重结晶 3 次,真空干燥,产率 40%,熔点为 46.5 ~ 47.5℃.

辛酸亚锡(Aldrich)用前重蒸,配成 0.01 g/mL 无水甲苯溶液;聚乙二醇单甲醚(mPEG)(Aldrich, $M_n = 2000$) 40℃真空干燥 72 h;D,L-丙交酯(LA)(Purac)在乙酸乙酯中重结晶两次,减压升华;9-NC(杭州海特医药有限公司,99.7%);THF(Merck,色谱纯)苊(Acros Organics)及其它试剂均为分析纯,购自北京化学试剂公司.

2 共聚物 PETLA 合成和载药胶束的制备

将一定比例的 TMC、LA、mPEG 加入干燥的聚合管中,反复减压充氮 6 次,氮气气氛下加入辛酸亚锡甲苯溶液, $[M]/[C] = 2000$,减压除溶剂后真空封管,于 160℃油浴锅中聚合 12 h.反应结束后,将聚合物粗产物溶于二氯甲烷并用无水乙醚沉淀,反复 3 次后在 30℃真空干燥至恒重,得到的共聚物为白色固体.

称取 2 mg 9-NC 和 20 mg PETLA 共聚物,溶于含 5 mL 二氯甲烷的具支试管中,得到澄清溶液,在氮气保护下于 60℃水浴中磁搅拌挥发二氯甲烷后,于 30℃真空干燥 24 h,得到均匀透明的聚

* 2007-05-28 收稿,2007-07-17 修稿 国家重点基础研究发展计划(973 计划,项目号 2005CB623903)资助项目;** 通讯联系人,

E-mail: zwgu@scu.edu.cn

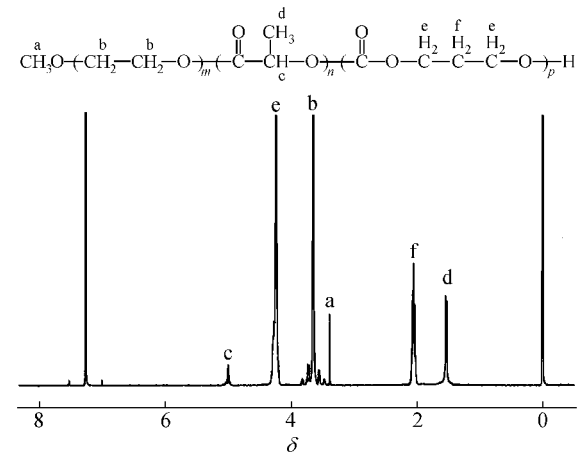


Fig. 2 ¹H-NMR spectrum of block copolymer PETLA2

具^[11]. 由于芘的荧光强度对周围环境极性变化具有强烈敏感性,在胶束形成过程中芘从溶液向疏水性胶束核迁移,因此选择芘作为荧光探针^[12-14].

测定系列浓度的共聚物芘溶液在 350 ~ 500 nm 范围内的荧光发射谱图,以波长 385 nm 和 373 nm 处的荧光强度之比(I_{385}/I_{373})对共聚物浓度作曲线(图 3),发现 I_{385}/I_{373} 在 $10^{-4} \sim 10^{-2}$ g/L 的浓度范围内发生突变,突变点对应的即为嵌段共聚物的 CMC. 对比具有不同疏水链段长度的嵌段共聚物的 CMC,可以看到随着疏水链段(分子量为¹H-NMR 测试结果)的增长,CMC 减小,如 PETLA1(疏水性链段分子量为 600)的 CMC 为 5×10^{-3} g/L,而 PETLA4(疏水性链段分子量为 4800)的 CMC 为 2.5×10^{-4} g/L,说明疏水链段较长的共聚物在水中发生自组装形成胶束的临界胶束浓度更低.

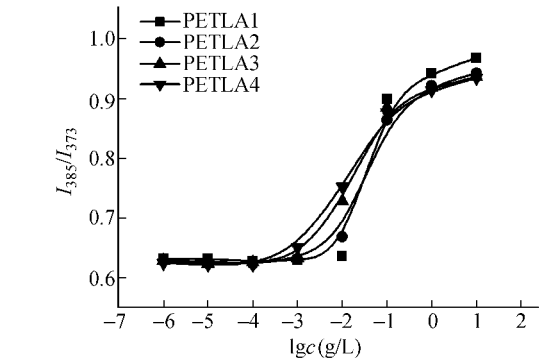


Fig. 3 Plots of the intensity ratio I_{385}/I_{373} from pyrene emission spectra versus concentrations (g/L) of copolymers from PETLA1 to PETLA4 in tri-distilled water

6 胶束的结构、尺寸及形貌表征

由于胶束是由多个嵌段共聚物分子缔合而成

的聚集体,呈缔合状态的胶束的分子量要比嵌段共聚物的分子量大得多,因此可以用 GPC 来表征胶束的形成^[15,16]. 图 4 是胶束 MT1 的水相 GPC 曲线,从图中可以看出在洗脱时间为 13.9 min 和 18.2 min 时分别有一个峰出现,其对应的重均分子量分别是 277500 和 2700(葡聚糖为标样). 由于胶束溶液中同时存在胶束聚集体和嵌段共聚物分子,因此大分子量对应的峰为胶束聚集体,分子量小的则为嵌段共聚物分子. 表 2 列出了空白胶束 MT2 和 MT3 的 GPC 测试数据. MT1、MT2、MT3 的亲水链段均为 mPEG2000,疏水性共聚物嵌段的分子量分别为 600,1900 和 3300(¹H-NMR 测试结果),由表中可以看到,胶束的分子量随着疏水嵌段链长的增加而增大.

嵌段共聚物胶束粒径的动态光散射(DLS)测试结果发现,胶束 MT1 到 MT3 的粒径由 32.7 nm 增加到 61.3 nm,胶束粒径随疏水性嵌段链长的增加而增加(表 2). 这可能是由于两种原因导致的:首先是疏水链段长度增加使胶束粒径加大,其次还可能由于疏水链段长度增加增强了共聚物间疏水作用,使构成同一个胶束的嵌段共聚物的数目增加,因而增大了胶束的尺寸. 这一结果与 GPC 测得的胶束分子量的变化趋势完全一致.

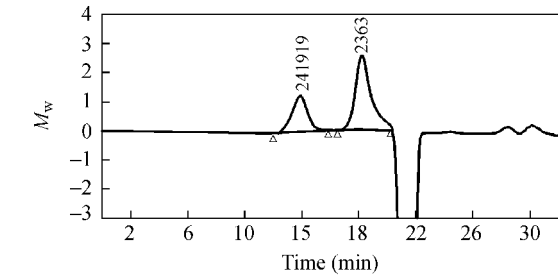


Fig. 4 GPC chromatograms of micelle MT1, from which peak molecular weight of MT1 and PETLA1 were 241919 and 2363 respectively

Table 2 Micelles molecular weight and size analysis through GPC and DLS

Micelles	M_w of micelle ^a	M_w of copolymer ^a	Diameter(nm) ^b
MT1	277500	2700	32.7 ± 5.3
MT2	462200	4000	48.3 ± 6.4
MT3	644800	5100	61.3 ± 10.2

^a GPC result; ^b DLS result

图 5 是胶束 MT1 冷冻干燥后重新溶于水,经磷钨酸负染后的透射电镜照片. 从图中可以看到,胶束近似为球形,呈良好的分散状态. 透射电镜观察到的胶束粒径为 30 ~ 40 nm,与表 2 中 DLS 测试

的结果基本一致.

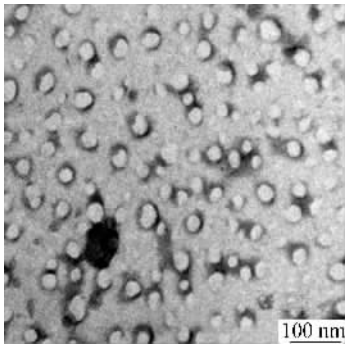


图 5 Transmission electron micrographs of micelle MT1 composed of copolymer PETLA1

7 负载 9-NC 的胶束体外药物释放行为研究

图 6 是载药量为 3% 的 MT 系列载药胶束 (MT1 ~ MT4) 在 PBS 中 (pH 7.4, 37℃) 的药物释放曲线. 从图中可以看到, 在最初释放的 2 h, 出现药物突释现象, 这是分布在胶束壳核界面或胶束外壳中的部分药物分子快速扩散到溶液中的结果. 2 h 以后的药物释放速率趋缓并接近零级恒速释放, 经 40 h 后, 释放的药物仅为 40% ~ 55%, 这可

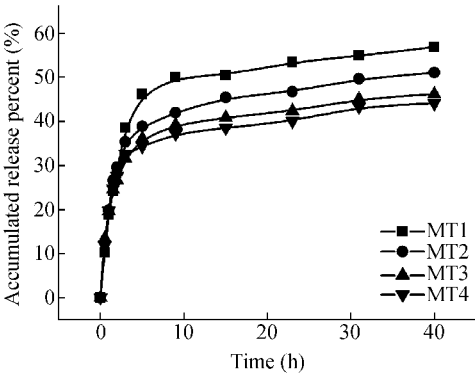


图 6 9-NC release profile from MT1 to MT4

能与 9-NC 药物分子和胶束内核较强的相互作用有关. 从图 6 中可以看到, 随着构成胶束的嵌段共聚物疏水链段长度的增加, 药物释放速度减慢, 其药物释放速率变化趋势为 MT1 > MT2 > MT3 > MT4. 这是因为疏水链段长的共聚物具有较强的疏水相互作用, 易形成尺寸较大的载药胶束, 药物分子从胶束内核扩散到周围溶液中需要更长的时间. 上述结果表明, 通过调整胶束疏水链段的长度能够实现 9-NC 的可控、恒速释放.

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MICELLIZATION AND DRUG CONTROLLED RELEASE OF AMPHIPHILIC METHOXY POLY(ETHYLENE GLYCOL)-*b*-POLY(D,L-LACTIDE-*co*-TRIMETHYLENE CARBONATE)

GAO Jieming¹, GUO Yingzhi², GU Zhongwei³, ZHANG Xingdong³

(¹ College of Material Science and Engineering, Southwest Jiaotong University, Chengdu 610031)

(² National Research Institute for Family Planning, Beijing 100081)

(³ National Engineering Research Center for Biomaterials, Sichuan University, Chengdu 610064)

Abstract Amphiphilic block copolymers (PETLA) composed of D,L-lactide, trimethylene carbonate (TMC) and methoxy poly(ethylene glycol) (mPEG) were synthesized with ring-opening copolymerization. Initiation of D,L-LA and TMC copolymerization with mPEG provided to manipulate the molecular weight of the hydrophobic block. Copolymer characterization with ¹H-NMR and gel permeation chromatograph (GPC) illustrated the final copolymer compositions were agreed with the theoretical values in feeding doses. Studies on micellization and drug controlled release behaviors of copolymer PETLA were performed with fluorescence spectroscopy, GPC, dynamic light scattering (DLS), transmission electron microscope (TEM), and ultraviolet spectroscopy (UV). Micelles of MT1 to MT4 were prepared from PETLA1 to PETLA4 respectively. Fluorescence technique with pyrene as hydrophobic probe was used to study the formation of core-shell type micelles. As a result, the critical micelle concentration (CMC) of PETLA was decreased from 5×10^{-3} g/L (PETLA1) to 2.5×10^{-4} g/L (PETLA4) with the increase of chain length in hydrophobic block while keeping constant corona-forming block. GPC characterization was used to describe the aggregation behavior of copolymers in selective solvent, by which the molecular weight of micelles was determined, and it was increased obviously from MT1 (277500 Dalton) to MT3 (644800 Dalton). Consistent to GPC results, DLS tests indicated the micellar diameters from MT1 to MT3 were increased from 30 nm to 60 nm. This would be related to the following reasons: the length of the hydrophobic block determined the space each chain required in the core, and the block copolymer with high hydrophobic length needed more core space to form larger micelles. TEM photograph illustrated that micelles MT1 had typical core-shell structure and regularly spherical morphology with the diameter of 30 ~ 40 nm after negative staining by phosphotungstic acid. Taking 9-nitro-20(s)-camptothecin (9-NC) as model drug, release profiles in vitro shown that the release behavior of 9-NC from micelles was in controlled manner and nearly in zero order after the initial burst release. The release rate decreased from MT1 to MT4, this was because the micelles composed of high hydrophobic composition could form large hydrophobic core and could encapsulate more drugs through strong hydrophobic interactions, and the incorporated drug would take relatively long time to overcome the hydrophobic interaction then diffuse across the polymer matrix to the medium. Based on the results given above, micelles with hydrophobic part composed of D,L-lactide and TMC were not only help to control the release behavior of hydrophobic drug, but also help to alleviate local inflammation response which was elicited by acid production biodegraded from poly(D,L-lactide). In conclusion, novel copolymer micelles could open new opportunities for the delivery of poorly soluble anticancer drugs.

Keywords Biodegradable, Methoxy poly(ethylene glycol)-*b*-poly(D,L-lactide-*co*-trimethylene carbonate), Micelle, Controlled release