CHITOSAN-NANOPARTICLES AS A DRUG DELIVERY SYSTEM FOR 5-FLUOROURACIL

by

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Chapter 5

HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF 5-FU IN RAT PLASMA AND ORGAN TISSUES

NANOPARTIKEL KITOSAN SEBAGAI SATU SISTEM PENGHANTARAN DRUG UNTUK 5-FLUOROURASIL

ABSTRAK

Sifat-sifat fizikokimia kitosan dicirikan dengan menentukan darjah deasetilasi (DD), purata kelikatan jisim molekul (Mv) kitosan, kelikatan, kestabilan, kelarutan, dan pH larutan kitosan. DD dan Mv kitosan masing-masing ialah 73 - 76%, dan 0.75 to 0.90 Daltons, manakala kelikatan 1% w/v kitosan di dalam 1 dan 5% v/v asid asetik masing-masing ialah 1.697± 0.023 dan 2.23±0.009 Pas. Selepas disimpan selama 10 hari, kelikatan kitosan dalam asid askorbik berkurang 76%, manakala kelikatan di dalam asid asetik, formik, laktik, maleik dan malik menurun dalam julat 21- 37%. Tambahan pula, kitosan lebih larut dalam asid asetik, formik, dan laktik daripada dalam asid maleik, askorbik dan malik. pH larutan 1% w/v kitosan di dalam pelbagai asid ialah dalam julat 1.91-2.62. Untuk tujuan penyediaan nanopartikel kitosan, pengaruh kepekatan kitosan, jenis dan kepekatan pengemulsi, jenis asid, kepekatan asid asetik, dan kaedah pencampuran diselidiki. Keputusan menunjukkan saiz nanopartikel berkadar langsung dengan kepekatan kitosan tetapi berkadar songsang dengan kepekatan pengemulsi. Kaedah pencampuran mengguna sonikator menghasilkan nanopartikel, manakala pencampuran mengguna pengacau magnetik menghasilkan mikrosfera. Pengoptimuman muatan drug dan kecekapan muatan dikaji menggunakan suatu rekabentuk faktorial 3^3 dengan mempelbagaikan amaun kitosan, 5-fluorourasil, dan glutaraldehid. Muatan drug dan kecekapan muatan masing-masing ialah 1.26 - 5.38% dan 10 - 30%. Muatan drug berkadar secara signifikan dengan amaun permulaaan 5-fluorourasil dan kitosan. Kecekapan muatan berkadar dengan kepekatan kitosan dan glutaraldehid. Kajian kinetik pelepasan drug

dilakukan mengguna alat Desaga dan dianalisa mengguna HPLC. Keputusan menunjukkan bahawa, kinetik pelepasan drug mengikuti pola dwifasa iaitu pelepasan drug yang cepat pada permulaan diikuti oleh pelepasan perlahan. Lebih kurang 35 hingga 49% drug dilepaskan dalam jangkamasa 1 jam pertama. Formulasi nanopartikel menggunakan 1.5% w/v kitosan, 35 mg 5-fluorourasil, dan 30 mg glutaraldehid dipilih untuk kajian *in vivo*, kerana muatan drug, kecekapan muatan, dan pelepasan *in vitro* adalah optimum. Kaedah HPLC yang mudah dan spesifik dibangunkan untuk memisahkan urasil, 5-fluorourasil, dan 5-bromourasil dalam plasma, jantung, buah pinggang, hati dan paru-paru. Penemuan semula mutlak 5-fluorourasil dalam plasma dan tisu organ adalah lebih besar daripada 89%. Nilai kejituan dan ketepatan analisa dalam satu hari dan di antara hari ialah kurang daripada 15%. Farmakokinetik dan biodistribusi nanopartikel mengandungi 5-fluorourasil dan injeksi 5-fluorourasil komersil dikaji menggunakan tikus jantan Sprague Dawley. Hasil bahagi luas bawah keluk (AUC), pemalar kadar penyingkiran (k_e), penyingkiran setengah hayat (t_e), masa untuk mencapai kepekatan puncak (T_{max}), dan kepekatan drug puncak (C_{max}) nanopartikel dengan larutan kawalan dalam plasma masing-masing ialah 0.67, 0.058, 17.00, 24, dan 0.16. Dalam organ tisu, AUC nanopartikel lebih tinggi daripada larutan kawalan. Hasil bahagi AUC nanopartikel dengan larutan kawalan ialah 2.04 - 8.78 dan mengikut urutan hati > jantung > buah pinggang > paru-paru.

Abstract

The physicochemical properties of chitosan were characterized by the determination of degree of deacetylation (DD), viscosity-average molecular mass (Mv), viscosity, stability, solubility, and pH of the chitosan solution. DD and Mv of chitosan were about 73 to 76% and 0.75 to 0.90 Daltons, respectively, where as the viscosity of 1% w/v chitosan in 1 and 5% v/v acetic acid were 1.697± 0.023 and 2.23±0.009 Pas, respectively. At the end of 10 storage days, viscosity of chitosan in ascorbic acid decreased by 76%, while in acetic, formic, lactic, maleic, and malic acids, viscosity decreased within a range of 21 – 37%. In addition, chitosan was more soluble in acetic, formic and lactic acids than maleic, ascorbic, and malic acids. The pH of 1% w/v chitosan in the various acids varied from 1.91 to 2.62. In order to formulate chitosan nanoparticles, the influence of chitosan concentrations, type and concentration of emulsifiers, type of acid, concentration of acetic acid, and mixing method were investigated. The results showed that the size of nanoparticles was directly correlated to the chitosan concentration but inversely associated with emulsifier concentrations. Mixing method by sonication produced nanoparticles, while magnetic stirring resulted in microspheres. The optimization of drug loading and loading efficiency was investigated using a 3^3 factorial designs by varying the amount of chitosan, 5-FU and glutaraldehyde. Drug loading and loading efficiency were 1.26 to 5.38% and 10 to 30%, respectively. Drug loading was significantly correlated with the initial amount of 5-FU and chitosan. Loading efficiency was correlated with the concentration of chitosan and glutaraldehyde. The drug release kinetics was studied using a Desaga apparatus and analyzed by a HPLC method. The

result showed a biphasic pattern consisting of a fast initial release, followed by a slower release. About 35 to 49% of drug was released within the first one hour. Nanoparticle formulation using 1.50% w/v chitosan, 35 mg of 5-FU, and 30 mg of glutaraldehyde were chosen for the *in vivo* study, since the drug loading, loading efficiency and *in vitro* release were optimum. A simple yet specific HPLC method was developed to separate uracil, 5-FU and 5-BU in plasma, heart, kidney, liver and lung. The absolute recoveries of 5-FU from the plasma and the organ tissues were higher than 89%. All the within-day and between-day precision and accuracy values were less than 15%. The pharmacokinetics and biodistribution of 5-FU loaded nanoparticles and a commercial 5-FU injection were studied using male Sprague Dawley rats. Area under the curve (AUC), elimination rate constant (k_e), elimination half-life (t_e), time to reach the peak concentration (T_{max}), and peak concentration of drug (C_{max}) quotients of nanoparticles to control solution in plasma were 0.67, 0.058, 17.00, 24, and 0.16, respectively. In the organ tissues, AUC of nanoparticles were higher than the control solution. AUC quotients of nanoparticles to control solution varied from 2.04 to 8.78 and followed the order of liver > heart > kidney > lung.