UNIVERSITI PUTRA MALAYSIA

PREPARATION OF ELLAGIC ACID, HIPPURIC ACID, PERINDOPRIL ERBUMINE AND CETIRIZINE NANOCOMPOSITES FOR DRUG DELIVERY

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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Chair: Prof. Mohd Zobir Bin Hussein, PhD

Faculty: Science

The administration of drugs designed to be given as a single dose rather than multiple doses has recently been made possible using controlled release formulation approach, so that the release of the drugs can be accomplished over long periods of time, enabling an almost constant level of the drug to be maintained in the bloodstream. Moreover, controlled release formulations increase the clinical efficacy of drugs. The introduction of drug nanocomposites as sustained release vehicles has provided a breakthrough in novel drug delivery systems in the field of pharmaceutical technology. Layered double hydroxides (LDHs) are widely used for this purpose. This study aimed at the synthesis of new controlled release formulation of ellagic acid, hippuric acid, perindopril erbumine and cetirizine hydrochloric acid via intercalation of the drug either into zinc-aluminium-, magnesium-aluminium-layered double hydroxides and zinc layered hydroxide, to increase the residence time in the body by sustained release and therefore increase the clinical efficacy.

Eighth nanocomposites were synthesized using zinc layered hydroxide, zinc-aluminium- and magnesium-aluminium-layered double hydroxides. Ellagic acid and hippuric acid were intercalated into zinc layered hydroxides to form ellagic acid, hippuric acid nanocomposites; EAN and HAN, respectively. Perindopril erbumine was also intercalated into zinc-aluminium-layered double hydroxides to form PZAC and PZAE nanocomposites by direct- and ion exchange-method, respectively. In addition, this drug was also intercalated into magnesium-aluminium-layered double hydroxides to form PMAN nanocomposite. In addition, cetirizine was intercalated into zinc layerd hydroxide, zinc-aluminium-layered double hydroxide and magnesium-aluminium-layered double hydroxide to form CETN, CTZAN and CTMAN nanocomposite, respectively.

The basal spacing for the nanocomposite is 10.4, 21.3, 21.7, 19.9, 21.98, 33.9, 31.9 and 31.2 Å for EAN, HAN, PZAE, PZAC, PMAE, CETN, CTZAN and CTMAN , respectively. The result coupled with molecular geometry calculation indicate that the spatial orientation of the drugs in the layered inorganic sheets was monolayer for EAN, HAN and PZAC nanocomposites; and as bilayers for PZAE, PMAE, CETN, CTZAN and CTMAN nanocomposites.

The release of the ellagic acid from its nanocomposite into aqueous solution of Na_3PO_4 and Na_2CO_3, at 38 hours was 94 % and 69 %, respectively. The release of the drugs from HAN, PZAE, PZAC, PMAE, CETN, CTZAN and CTMAN nanocomposites at pH 7.4 is 3780, 1000, 1263, 5000, 4980, 600 and 2980 minutes, respectively, compared to 1260, 1100, 1020, 1000, 4320, 600 and 750 minutes, respectively at pH 4.8. This result indicates sustained release of the drugs from their

respective nanocomposites, and therefore these nanocomposites have good potential to be used as controlled-release formulation of the drugs.

In vitro bioassay study for EAN nanocomposite, the result showed that EAN has a mild effect on the hepatocytes cells, similar to its counterpart, the free EA. HAN has synergistic properties with tamoxifen toward a HepG2 cells line, with an IC_{50} value of 0.35 compared to hippurate. In the antiproliferative assay, the ratio of viable cells account for cells treated by the combination of tamoxifen with HAN to untreated cells was sharply reduced to 66 % and 13 % after 24 and 72 hours, respectively.

The angiotensin converting enzyme (ACE) inhibition activity of perindopril in PZAE, PZAC and PMAE nanocomposites was determined in vitro. The three formulations shows inhibition with 70.6, 70.1 and 55.4 % for PMAE, PZAE and PZAC nanocomposites, respectively. The MgAl-LDH shows mild potent ACE inhibitory activity with 5.6 % decreased in ACE activity, while the ZnAl-LDH did not show any effect toward ACE enzyme.

Release of histamine from RBL-2H3 cells was found to be more sensitive to the intercalated cetirizine in the CETN nanocomposite compared to its free counterpart with inhibition of 56 % and 29 %, respectively at 62.5 ng/ml. The inhibition of histamine by the free cetirizine is higher than the intercalated cetirizine into CTZAN and CTMAN nanocomposites.

This study showed that the formation of organic-inorganic nanocomposite materials of ellagic acid, hippuric acid, perindopril erbumine and cetirizine as organic guests and zinc-aluminium-layered double hydroxide, magnesium-aluminium-layered

double hydroxides and zinc-layered hydroxide as hosts can be successfully accomplished. All the nanocomposites show controlled release property of the organic guests, the drug actives, and therefore are useful for drug delivery systems.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENYEDIAAN NANOKOMPOSIT ASID ELAGIK, ASID HIPPURIK, PERINDOPRIL ERBUMIN DAN SETRIZIN UNTUK PENGHANTARAN UBAT

Oleh

SAMER HASAN AHMAD HUSSEIN AL ALI

September 2012

Pengerusi: Profesor Mohd Zobir bin Hussein, PhD

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Penyampaian ubat yang direka untuk diberikan melalui satu dos berbanding beberapa dos telah direalisasikan melalui formulasi pelepasan terkawal. Dengan itu, pelepasan ubat dapat dilakukan dalam tempoh yang panjang dan seterusnya membolehkan kadar ubat yang malar dalam saluran darah. Selain daripada itu, formulasi pelepasan terkawal turut meningkatkan keberkesanan klinikal ubat. Pengenalan nanokomposit ubat sebagai agen pelepasan berterusan adalah merupakan suatu kaedah penyampaian ubat yang novel dalam bidang teknologi farmaseutikal. Hidroksida berlapis ganda (LDH) telah digunakan secara meluas untuk tujuan tersebut. Kajian ini adalah merangkumi sintesis formulasi pelepasan terkawal bagi ubat-ubatan seperti asid elagik, asid hipurik, perindopril erbumin dan setrizin melalui interkalasi ke dalam zink-aluminium-, magnesium-aluminium-LDH dan hidroksida belapis zink. Kaedah yang digunakan adalah bertujuan untuk meningkatkan tempoh kehadiran ubat di dalam badan melalui pelepasan berterusan daripada nanokompositnya.

Dalam kajian ini, lapan nanokomposit telah disintesis menggunakan hidroksida berlapis zink, zink-aluminium- dan magnesium-aluminium-LDHs sebagai perumah.

Asid elagik dan asid hipurik telah diinterkalasi ke dalan hidroksida berlapis zink bagi menghasilkan nanokomposit asid elagik (EAN) dan nanokomposit asid hipurik (HAN). Perindopril erbumin telah diinterkalasi ke dalam zink-aluminium LDH melalui kaedah terus dan kaedah pertukaran ion masing-masing bagi menghasilkan PZAC dan PZAE. Selain daripada itu, ubat ini turut diinterkalasi ke dalam magnesium-aluminium LDH untuk menghasilkan nanokomposit PMAN. Seterusnya setrizin telah juga diinterkalasikan ke dalam hidroksida berlapis zink, zink-aluminium- dan magnesium-aluminium-LDH masing-masing bagi penghasilan nanokomposit CETN, CTZAN dan CTMAN.

Jarak basal bagi semua nanokomposit EAN, HAN, PZAE, PZAC, PMAE, CETN, CTZAN dan CTMAN adalah 10.4, 21.3, 21.7, 19.9, 21.98, 33.9, 31.9 dan 31.2 Å. Keputusan ini bersama dengan pengiraan geometri molekul menunjukkan bahawa orientasi ruang bagi tetamu dalam nanokomposit EAN, HAN dan PZAC adalah ekalapis sementara tetamu dalam nanokomposit PZAE, PMAE, CETN, CTZAN dan CTMAN mempunyai orientasi ruang yang terdiri daripada dua lapisan.

Pelepasan asid elagik daripada nanokomposit selepas 38 jam adalah 94% dalam larutan akueous Na_3PO_4 dan 69% bagi Na_2CO_3. Pelepasan ubat pada pH 7.4 daripada nanokomposit HAN, PZAE, PZAC, PMAE, CETN, CTZAN dan CTMAN berlaku masing-masing selepas 3780, 1000, 1263, 5000, 4980, 600 dan 2980 minit. Manakala pada pH 4.8, pelepasan ubat dari semua nanokomposit yang tersenarai di atas masing-masing berlaku selepas 1260, 1100, 1020, 1000, 4320, 600 dan 750 minit.

Kajian menggunakan bioassay in vitro bagi nanokomposit asid elagik, EAN menunjukkan bahawa ianya memberikan kesan yang sedikit kepada sel hepatosit iaitu hampir menyamai asid elagik bebas, EA. Manakala nanokomposit asid hipurik,

HAN mempunyai ciri-ciri bersinergi dengan tamoxifen terhadap sel-sel HepG2 dengan nilai IC_{50} 0.35 berbanding dengan hippurat. Dalam assay antiproliferative, nisbah sel hidup adalah sama dengan sel yang telah dicampur dengan kombinasi tamoxifen dan HAN berbanding sel bebas. Nisbah sel hidup ini berkurangan sehingga 66% selepas 24 jam manakala jumlah pengurangan adalah 13% selepas 72 jam.

Penghalangan aktiviti enzim penukaran angiotensin (ACE) oleh perindopril bagi nanokomposit PZAE, PZAC dan PMAE telah ditentukan secara in vitro. Ketiga-tiga nanokomposit iaitu PMAE, PZAE and PZAC masing-masing menghalang aktiviti enzim ACE sebanyak 70.6, 70.1 dan 55.4%.

Pelepasan histamin dari sel RBL-2H3 didapati lebih sensitif terhadap setrizin yang diinterkalasi di dalam nanokomposit CETN berbanding setrizin bebas. Peratusan halangan pembebasan histamin pada kepekatan 62.5 ng/ml bagi nanokomposit CETN dan setrizin bebas masing-masing adalah 56% dan 29%. Manakala, kadar halangan pembebasan histamin oleh setrizin bebas adalah lebih tinggi berbanding setrizin yang diinterkalasi ke dalam nanokomposit CTZAN dan CTMAN.

Kajian ini menunjukkan bahawa pembentukan bahan nanokomposit organik-inorganik daripada sebatian organik seperti asid elagik, asid hipurik, perindopril erbumin dan setrizin dengan hos inorganic zink-aluminium LDHs, magnesium-aluminium LDHs dan hidroksida berlapis zink telah dapat dilaksanakan dengan jayanya. Semua nanokomposit tersebut menunjukkan ciri-ciri pelepasan terkawal bahan organik bagi bahan aktif ubatan, dan ini menjadikan ianya berguna bagi sistem penyampaian ubat.

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I certify that a Thesis Examination Committee has met on 28 September 2012 to conduct the final examination of Samer Hasan Ahmad Hussein Al Ali on his thesis entitled "**PREPARATION OF ELLAGIC ACID, HIPPURIC ACID, PERINDOPRIL ERBUMINE AND CETIRIZINE NANOCOMPOSITES FOR DRUG DELIVERY**" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of **Doctor of Philosophy**. The members of the Supervisory Committee were as follows:

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DECLARATION

I declare that the thesis is my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

SAMER HASAN AHMAD HUSSEIN AL ALI

Date:23 September 2012

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