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FORMATION AND CHARACTERIZATION OF NANOEMULSION SYSTEM CONTAINING HYDROCORTISONE FOR TRANSDERMAL APPLICATION

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By

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FORMATION AND CHARACTERIZATION OF NANOEMULSION SYSTEM CONTAINING HYDROCORTISONE FOR TRANSDERMAL APPLICATION

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Chairman : Professor Mahiran Basri, PhD
Faculty : Science

Nanoemulsion is one of the most efficient dispersed delivery systems of particle size ranging in nano size. There are mainly two types of nanoemulsions; oil in water (O/W) and water in oil (W/O) nanoemulsion. O/W nanoemulsions were formulated to deliver hydrocortisone drug to the target site. The compositions were selected from the constructed phase diagrams. The addition of solvents gave a larger isotropic and homogeneous region in the ternary phase diagrams. Initially three type of solvents were selected, namely isopropanol (IPA), ethyl acetate (EA) and ethanol (EtOH). However due to the low solubility of hydrocortisone in ethyl acetate, it is not included in the phase diagram study. Phase diagrams of PKOEs/Lipoid S75:Tween 20 (60:40)/Water, PKOEs/IPA:Lipoid S75 (1:1)/Water:Tween 20 (60:40) and PKOEs/EtOH:Lipoid S75 (50:50)/Water:Tween 20 (60:40) systems were constructed at room temperature. It was observed that the PKOEs/IPA:Lipoid S75 (1:1)/Water:Tween 20 (60:40) and PKOEs/EtOH:Lipoid S75 (50:50)/Water:Tween 20 (60:40) systems showed improved solubility of water to give larger isotropic region compared to the PKOEs/Lipoid S75:Tween 20 (60:40)/Water system.

A total of 9 compositions from the homogeneous region of the PKOEs/Lipoid S75:Tween 20 (60:40)/Water system were chosen because of the low surfactant concentration. The emulsions were then subjected to mechanical stirring for 4 hours to produce nano-sized emulsions which were then tested for accelerated stability (centrifugation). Nanoemulsion at point C (NEC) which exhibited highest stability was chosen for further studies. The influence of solvents and surfactant concentration to the stability and physical behavior of the palm based nanoemulsion was studied. The stability of the system was evaluated by measuring the particle size and zeta potential of nanoemulsion at room temperature over a period of 3 m. The mean droplet size for freshly prepared negatively charged nanoemulsions showed slight deviation. A decrease particle size was observed in nanoemulsions after solvent evaporation while particle size of nanoemulsions with different solvent concentration showed no significant difference.

As for the positively charged nanoemulsions, the mean particle size of formulations decreased as the concentration of phytosphingosine was increased because phytosphingosine acts as a cosurfactant. All formulations except NEC[IPA:Lipoid (1:1)] SE and NEC[EtOH:Lipoid (1:1)] SE showed no significant difference in particle size. All formulations remain stable without visible phase separation in three different temperatures (5 ℃, room temperature and 45 ℃) for a period of 3 months. These findings showed that the presence of solvents increased the nanoemulsion stability thereby prolong the shelf life of the formulation.
DSC thermograms showed no hydrocortisone peak for all the nanoemulsion samples prepared suggesting that hydrocortisone was dispersed in the nanoemulsions in an amorphous or non-crystalline state. The particle size measured in Transmission Electron Microscopy (TEM) was consistent with the size obtained using photon correlation spectroscopy. There was increment in size in the positively charged nanoemulsions as opposed to the negatively charged nanoemulsions. All the formulations were stable after undergoing heat-cool cycles, storage at 5 ℃, room temperature and 45 ℃ for more than 12 months. The good stability was due to the high negative and positive surface charged induced by phytosphingosine.

Biological activities of nanoemulsions were investigated using *in vitro* microbiological test. The results showed no bacterial growth in all nanoemulsions. This implied that the nanoemulsions have an antimicrobial effect against *Escherichia coli*. *In vitro* drug permeation results showed that the prepared nanoemulsions gave better drug release as compared to marketed hydrocortisone cream. This result further supports the claims of solvents as a penetration enhancer. Furthermore, histopathological studies illustrated that the formulated nanoemulsions did not cause any skin irritation, indicating that it is safe for human use. In conclusion, the nanoemulsion formulation is a promising vehicle for the delivery of hydrocortisone transdermally.

Abstrak tesis dikemukan kepada Senat Universiti Putra Malaysia sebagai memenuhi syarat untuk ijazah Master Sains

PEMBENTUKAN DAN PENCIRIAN SISTEM NANOEMULSI YANG MENGANDUNGI HIDROCORTISONE UNTUK PENGHANTARAN MELALUI KULIT

Oleh

STEPHANIE SHARON DA COSTA

November 2013

Pengerusi : Profesor Mahiran Basri, PhD
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Nanoemulsi adalah salah satu sistem penyampaian yang lebih berkesan disebabkan saiz zarah yang bersaiz nano. Terdapat dua jenis nanoemulsi iaitu minyak dalam air (O/W) dan air dalam minyak (W/O) nanoemulsi. Komposisi telah dipilih dari gambar rajah fasa yang dibina. Penambahan pelarut memberikan bahagian isotropik dan homogen yang lebih besar dalam gambar rajah tiga fasa. Pada mulanya tiga jenis pelarut telah dipilih iaitu isopropanol (IPA), etil asetat (EA) dan etanol (EtOH). Walau bagaimanapun disebabkan oleh kelarutan hidrocortisone yang rendah dalam etil asetat, ia tidak termasuk dalam kajian gambarajah tiga fasa. Gambar rajah tiga fasa bagi sistem PKOEs/Lipoid S75: Tween 20 (60:40)/Air, PKOEs/IPA: Lipoid S75 (1:1)/Air: Tween 20 dan PKOEs/EtOH: Lipoid S75 (1:1)/Air: Tween 20 telah dibina pada suhu bilik. PKOEs/IPA: Lipoid S75 (1:1)/Air: Tween 20 dan PKOEs/EtOH: Lipoid S75 (1:1)/Air: Tween 20 sistem menunjukkan keterlarutan air yang lebih baik lalu memberi bahagian isotropi yang lebih besar berbanding dengan sistem PKOEs/Lipoid S75: Tween 20 (60:40)/Air.

Sebanyak 9 komposisi dari rantau homogen dari sistem PKOEs/Lipoid S75: Tween 20 (60:40)/Air telah dipilih kerana kepekatan surfaktan yang rendah. Emulsi kemudian terhasil melalui teknik pengacauan mekanikal untuk 4 jam dengan tujuan menghasilkan emulsi bersaiz nano yang kemudian diuji untuk kestabilan melalui centrifugasi. Nanoemulsi pada titik C (NEC) yang mempamerkan kestabilan tertinggi telah dipilih untuk kajian selanjutnya. Pengaruh pelarut dan kepekatan surfaktan kepada kestabilan dan sifat fizikal nanoemulsi berasaskan sawit telah dikaji. Kestabilan sistem itu dinilai dengan mengukur saiz zarah dan keupayaan zeta nanoemulsi pada suhu bilik dalam tempoh 3 bulan. Nanoemulsi bercas negatif yang baharu disediakan menunjukkan terdapatnya sedikit sisihan bagi min saiz titisan. Penurunan saiz zarah diperhatikan dalam nanoemulsi

selepas penyejatan pelarut manakala saiz zarah nanoemulsi dengan kepekatan pelarut yang berbeza tidak menunjukkan perbezaan yang ketara.

Manakala, bagi nanoemulsi yang bercas positif, min saiz zarah formulasi menurun apabila kepekatan fitostingosin meningkat kerana fitostingosine memainkan peranan sebagai kosurfaktan. Semua formulasi kecuali NEC[IPA: Lipoid (1:1)]SE dan NEC[EtOH:Lipoid (1:1)]SE menunjukkan tiada perbezaan yang ketara dalam saiz zarah. Semua formulasi kekal stabil tanpa pemisahan fasa pada tiga suhu yang berbeza (5 °C, suhu bilik dan 45 ℃) selama 3 bulan. Penemuan ini menunjukkan bahawa kehadiran pelarut meningkatkan kestabilan nanoemulsion sekali gus memanjangkan jangka hayat formulasi.

Selain itu, termogram DSC tidak menunjukkan puncak hidrokortison untuk semua sampel nanoemulsi yang disediakan. Ini adalah kerana hidrokortison telah tersebar di dalam nanoemulsi dalam keadaan yang amorfus dan bukan hablur. Saiz titisan diukur dengan mikroskop elektron penghantaran (TEM) selaras dengan saiz yang diperoleh menggunakan spektroskopi korelasi foton (DSC). Bagi nanoemulsi bercas positif mikrograf menunjukkan bahawa terdapat peningkatan dalam saiz dalam nanoemulsi bercas positif berbanding dengan nanoemulsi bercas negatif. Semua formulasi stabil selepas menjalani kitaran sejuk panas, penyimpanan pada 5 ℃, suhu bilik dan 45 ℃ selama lebih daripada 12 bulan. Kestabilan yang baik adalah disebabkan oleh cas negatif dan positif yang tinggi pada permukaan titisan.

Aktiviti biologi nanoemulsi telah disiasat melalui ujian mikrobiologi *in vitro*. Keputusan menunjukkan tiada pertumbuhan bacteria dalam semua nanoemulsi. Ini membuktikan bahawa nanoemulsi mempunyai kesan anti mikrobial terhadap bakteria, *Escherichia coli*. Begitu juga, dalam keputusan penyerapan dadah *in vitro* yang menunjukkan bahawa nanoemulsi bersedia memberikan pelepasan dadah yang lebih baik berbanding dengan krim hidrokortison di pasaran. Keputusan ini menyokong yang mengatakan pelarut adalah penggalak penembusan. Tambahan pula, kajian histopathological digambarkan bahawa nanoemulsi yang dirumus tidak menyebabkan sebarang iritasi kulit. Ini menunjukkan bahawa nanoemulsi adalah selamat untuk kegunaan manusia. Walau bagaimanapun ujian lanjut diperlukan untuk mewajarkan tuntutan tersebut. Kesimpulannya, formulasi nanoemulsi boleh menjadi kaedah penghantaran hidrokortison yang berkesan melalui kulit.

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I certify that a Thesis Examination Committee has met on 25th November 2013 to conduct the final examination of Stephanie Sharon Da Costa on her thesis entitled "STEROIDAL ANTIINFLAMMATORY DRUG NANOEMULSION SYSTEM FOR EFFECTIVE TRANSDERMAL APPLICATION" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the University Putra Malaysia [P.U.(A) 106}] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.
Members of the Thesis Examination Committee were as follows:

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Date: 17 February 2014

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of Supervisory Committee were as follows:

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Date: 17 February 2014

DECLARATION

Declaration by graduate student

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