ABSTRACTS

PST

BANGKOK 2019 THAILAND

The International Conference and Exhibition on Pharmaceutical Sciences and Technology 2019

"Pharmaceutical Engineering and Pharmaceutical Sciences for Human Health"

18-19 June 2019 ▪ The Ambassador Bangkok Hotel, Bangkok, Thailand

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In vitro antibacterial activity of hydrogels containing tamarind seed husk extracts against *Propionibacterium acnes*

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Abstract. *Propionibacterium acnes (P. acnes)* is one of skin bacteria that induce acne comedone formation. Previous studies reported that *P. acnes* was susceptible to alcoholic seed husk extract of *Tamarindus indica* L. *(T. indica)*, no studies have been reported about the susceptibility of water extract from tamarind seed husk to *P. acnes.* Therefore, the objectives of this investigation were to explore the antibacterial activity of water and ethanolic seed husk extracts from *T. indica* (wTSH and eTSH) against *P. acnes* and to compare antibacterial activity of hydrogels containing wTSH and eTSH extracts against *P. acnes*. wTSH and eTSH extracts were reddish brown powder, and oligomeric proanthocyanidin was identified by UV-Vis spectroscopy and high performance liquid chromatography as the main ingredient in the extracts. wTSH and eTSH extracts showed the average of total phenolic compounds of 408.3±0.01 and 385.5±0.02 mg GAE/g and exhibited very strong free radical scavenging ability by DPPH assay with IC50 value of 19.6±0.2 µg/ml and 18.8±0.2 µg/ml, respectively. In addition, wTSH and eTSH extracts showed good activity against *P. acnes*. The minimum inhibitory concentration (MIC) of wTSH and eTSH extracts was 500 and 250 µg/ml, and the minimum bactericidal concentration (MBC) of both extracts was 500 µg/ml. In conclusion, the hydrogels which composed of 0.1% wTSH or eTSH extracts, 2% HPMC, 15% glycerin and 1% paraben concentrate, exhibited weak acid with pH 5±2, good physical stability, i.e. good spreadability with pseudoplastic flow, and also exhibited noticeable anti *P. acnes* activity in fluorescent resazurin assay after storage in the accelerated conditions for 3 months.

Keywords: *P. acnes*, *T. indica*, tamarind seed husk, hydrogel

Extraction method of protein and insulin-like growth factor-1 from deer antler velvets for skin rejuvenation

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Abstract. Deer antler velvets (DAVs) contain many growth factors and protein. To extract growth factor from DAV, the suitable method and material properties have to investigate. The objective of this research was to improve the growth factor content in DAV extract for using as a skin rejuvenation compound. Different extraction methods (such as ethanol extract, probe sonication and precipitation method) and material preparations (such as fresh, dried by freeze drier and dried by hot air oven with and without gramma ray) were performed to evaluate the total protein and insulin-like growth factor-1 (IGF-1). The suitable condition was choose to determine the antioxidant activity and effect on the skin properties (such as in vitro skin permeation and in vivo human studies). For the results, fresh DAV extracted by probe sonication method provided the significantly highest total protein (586.31 + 48.17 mg/g) and IGF-1 (31.32 + 10.55 ng/g) contents. At the concentration of 2,000 µg/ml, this extract was completely dissolved in water and exhibited the antioxidant potential nearly 50% inhibition. For skin permeation at 24 h, the skin treated with DAV extract showed 3.83 + 2.04% of protein permeated through skin. The skin elasticity and hydration significantly increased after applying DAV extract for 28 days. In conclusion, the DAV extract by using fresh DAV and probe sonication method exhibited high IGF-1 and protein content as well as an antioxidant potential, leading to improve the skin properties. This extract might play an important role in the skin rejuvenation product.

Keywords: deer antler velvets, extraction method, Insulin-like growth factor-1, protein, skin

Bioequivalence study of olanzapine 5 mg orally disintegrating tablets formulations in healthy Thai volunteers under fasting conditions

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Abstract. A comparative randomized, single dose, two-way crossover, open label study was carried out to assess bioequivalence and tolerability of test (ZOLAN GPO®) and reference (Zyprexa Zydis®) products of olanzapine 5 mg orally disintegrating tablets for interchangeability in the same quality and safety. Twenty-six male and female volunteers were enrolled in and completed the study. Blood samples were collected at predefined time points over 72 hours after oral administration under fasting conditions. Plasma concentrations of olanzapine were determined using a validated liquid chromatography tandem mass spectrometry, capable to quantify olanzapine in the range of 0.098-40.896 ng/mL. The pharmacokinetic parameters were calculated for test and reference products using non-compartmental analysis. Bioequivalence between the products was determined by calculating 90% confidence intervals (90% CIs) for the ratios of Cmax and AUC truncated at 72 hours (AUC0−72) for the test and reference products using log-transformed data. Pharmacokinetics of olanzapine were in agreement with the literature data. No significant difference was found based on ANOVA; 90% CI (92.67–106.38% for Cmax and 95.36–103.81% for AUC0−72) of test/reference ratio for these parameters were found within bioequivalence acceptance range of 80.00-125.00%. Both treatments were well tolerated, and none of subjects developed any serious adverse events. It can be concluded that the ZOLAN GPO® was bioequivalent to Zyprexa Zydis® and can be used interchangeably.

Keywords: olanzapine, ODT, bioequivalence, pharmacokinetics, LC-MS/MS

Bioequivalence evaluation of two clopidogrel 75-mg tablet formulations (Clopidogrel GPO® and Plavix®) in healthy Thai volunteers

Vipada Khaowroongrueng[1, a *], Chutima Manamuti[1, b], Jaturavit Vattanarongkup[1, c], Polsak Teerawonganan[1, d], Mariam Duereh[1, e], Ekawan Yoosakul[1, f], Charinthon Seeduang[1, g], Bancha Chuasuwan[1, h], Isariya Techatanawat[1, i], Busarat Karachot [1, j], Nuntakan Suwanpidokkul[1, k] and Porranee Puranajoti[2]

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Abstract. Clopidogrel is a P2Y12 platelet inhibitor indicated for acute coronary syndrome and recent MI, recent stroke, or established peripheral arterial disease. The Government Pharmaceutical Organization, Thailand has developed a generic product of clopidogrel at a reduced cost to serve as an alternative product for physicians and patients. A comparative, two-way crossover, open-label bioequivalence study was conducted to compare the rate and extent of absorption of clopidogrel from the test product (Clopidogrel GPO®) with that of the reference product (Plavix®), as well as to evaluate the safety of the formulations in healthy subjects. One hundred and three male and female subjects were enrolled in and completed the study. Each subject randomly received a single dose of the assigned formulation after overnight fasting in each period, separated by 7-day washout period. After dosing, serial blood samples were collected at predefined time points for a period of 24 hours. Plasma harvested from blood was analyzed for clopidogrel in the range of 20.219 – 8022.433 pg/mL using a validated liquid chromatography-mass spectrometry analytical method. Pharmacokinetic parameters including AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, T_{max}, $T_{1/2}$, and λ_Z were determined from plasma concentration-time profiles and the primary pharmacokinetic parameters of both formulations were statistically compared. The analysis of variance (ANOVA) did not show any significant difference between the two formulations and the 90% confidence intervals for the ratio of log-transformed AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} fell within the acceptable range (80–120%) for bioequivalence. Both treatments were well tolerated and no serious adverse events were reported. It can be concluded that two clopidogrel 75-mg tablets were bioequivalent based on statistical inferences.

Keywords: clopidogrel, bioequivalence, pharmacokinetics

Porous hydroxyapatite/ chitosan/ carboxymethyl cellulose scaffolds with tunable microstructures for bone tissue engineering

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Abstract. Bone tissue engineering is an alternative approach to generate bone using biomaterials and cells. Hydroxyapatite (HA) has good biocompatibility, osteoinductivity, and osteoconductivity. However, it has limited utility due to poor mechanical properties and slow degradation rate. To improve the mechanical properties and to modify the degradation profile, hydroxyapatite was tethered in chitosan (CS) and carboxymethyl cellulose (CMC) complex. Gelatin was incorporated to promote cell attachment and polyvinyl alcohol (PVA) was used to improve the mechanical strength. The physico-mechanical and biological properties of these scaffolds were investigated. Fourier transform infrared (FTIR) analysis and X-ray diffraction (XRD) showed the incorporation of hydroxyapatite in polymer matrix. The scaffolds had density, compressive strength, and Young's modulus in the range of 0.24-0.30 g/cm^3, 0.028-0.035 MPa, and 0.178-0.560 MPa, respectively. The scaffolds had the porosity of 69-91 percent. Higher content of PVA decreased the porosity of the scaffolds. Scanning electron microscope showed porous microstructure with pore size in the range of 60-183 μm. *In vitro* test on MC3T3-E1 preosteoblast cells showed negligible cytotoxicity of scaffolds. The data suggested that the HA/CS/CMC/gelatin/PVA scaffold has potential applications in bone tissue engineering.

Keywords: hydroxyapatite, chitosan, carboxymethyl cellulose, scaffold, bone tissue engineering

Antimicrobial activity of the prepared emulgel containing the combined crude extracts of *Psidium guajava* (Guava) and *Cassia alata* (Akapulko) leaves

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Abstract. Medicinal compounds from plants have a key role in the sustentation of human health since the ancient times. *Psidium guajava* Linn. (Family *Myrtaceae*) and *Cassia alata* Linn. (Family *Leguminosae*) are traditionally used to treat various skin ailments cause by certain bacteria and fungi. These two selected plants are two of the top ten medicinal plants endorsed by the Department of Health, Philippines. This study aims to determine the antimicrobial activity of the crude extracts from *Psidium guajava* and *Cassia alata* leaves in a prepared emulgel. The dried leaves of *P. guajava* and *C. alata* were powdered. For crude extracts, the powdered samples were percolated using 2L of 80% ethanol. In the removal of residual solvents, filtrates were evaporated to dryness by a rotary evaporator with a temperature maintained at 78˚C. For the defatted extracts, ground samples of guava and akapulko were soaked for 24h with 1.5L of hexane and they were percolated. The residues were collected, dried and were divided into two, first residue was used for the extraction with ethyl acetate and the other with 80% ethanol by the same procedure that carried out for defatting with hexane. Ethanol solvent was removed by rotary evaporator maintained at 78˚C and ethyl acetate solvent was removed by treating at 40˚C in an oven. All extracts were lyophilized. In the screening of extracts, each 20% w/v concentration of the solvents were evaluated through susceptibility testing using the agar well diffusion method against the four most common skin pathogens, namely, *Staphylococcus aureus, Pseudomonas aureginosa, Trichophyton rubrum,* and *Candida albicans*. Guava extracts were tested against the bacteria, and Akapulko extracts were tested against the fungi. The crude extracts exhibiting the highest antimicrobial activity were used to prepare the antimicrobial emulgel with different concentrations i.e 5%w/v, 20%w/v and 40%w/v. The emulgel were characterized through physical examination, determination of their pH, rheological studies, and spreadability testing. The defatted ethylacetate extract of the two plants showed the largest zone of inhibition, thus suggesting the highest antimicrobial activity. These two extracts were combined and used for the preparation of the emulgel. The parameters in the characterization of the emulgel such as, pH, viscosity, and spreadability were found to be within the limits. The optimized preparation of the emulgel containing the combined crude extracts of *Psidium guajava* and *Cassia alata* leaves have demonstrated a better antimicrobial activity. The effectiveness of the plant extracts was almost the same with the conventional antimicrobial agent, Combiderm® cream (Clotrimazole 1%, beclometasone dipropionate 0.025%, gentamicin sulfate 0.1%).

Keywords: *Psidium guajava, Cassia alata*, antimicrobial, topical emulgel

Formulation of a pediculicidal shampoo from *Annona squamosa* and *Azadirachta indica* fixed oils

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Abstract: Headlice infestation has always been a public health issue to children affecting their health and self-confidence. This will further worsen if left untreated which may cause other hair problems such as dandruff, itchiness, skin irritation and wounds due to frequent scratching. Various pediculicidal products are available in the market and most of them contain toxic properties and frequent use often lead to resistance. A good alternative treatment is extracted oils of *Annona squamosa* and *Azadirachta indica*. This urged the researchers to formulate an organic-based shampoo from the fixed oils of *A. squamosa* and *A. indica* for the treatment of headlice infestation and dandruff. Fixed oils were extracted using soxhlet apparatus and five different concentrations were optimized for its antibacterial and pediculicidal activity. Optimized concentration was used for shampoo formulation and further analyzed for its cleaning and pediculicidal action using filter paper diffusion bioassay. Among the treatment groups, F5 (1.25:0.75) is the optimized concentration and comparable to commercialized 1% permethrin and quassinoids. This showed that the formulated pediculidical shampoo from *A. squamosa* and *A. indica* fixed oils is a good alternative for treating headlice infestation.

Keywords: *Annona squamosa, Azadirachta indica,* dandruff, fixed oil, pediculicidal shampoo

Mixed solvent-lauric acid solvent-exchange induced *in situ* forming gel

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Abstract. Designing lauric acid (L) solvent-exchange induced *in situ* forming gel (ISG) was conducted in this study by using mixed solvents (*N*-methyl pyrrolidone (NMP), 2-pyrrolidone (PYR) and dimethyl sulfoxide (DMSO) at different ratios. The pH, density, viscosity, matrix formation, contact angle/surface tension and antimicrobial activities of 50% w/w L in mixed solvents were determined. The pH and density of solvent decreased apparently with L addition. More amount of PYR in the solvent mixture increased the density and viscosity. High viscous manner of PYR resulted in the high surface tension and contact angle. The solvent mixture of NMP/PYR promoted a higher contact angle than the other mixed solvent systems. L in DMSO, PYR or solvent mixture with high ratio of DMSO or PYR transformed into matrix-like rapidly while those in NMP or high ratio of NMP had slower matrix formation after exposure to aqueous fluid. NMP and PYR showed antimicrobial activities effectively against all test microbes while L matrix retarded their activities. The mixed solvent concept is interesting to use for designing ISG comprising L as matrix former to prolong the drug release.

Keywords: mixed solvent, fatty acid, *in situ* forming system, matrix, phase inversion, *N*-methyl pyrrolidone, 2-pyrrolidone, dimethyl sulfoxide

Evaluation of rice bran as a disintegrant in paracetamol tablet

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Abstract. Rice is the main food of Thai people. Rice milling process creates plenty supply of rice bran as by-product. Rice bran comprises mostly hemicellulose and cellulose which can absorb water and swell. In this study, the properties of Khao Dawk Mali 105 rice bran were evaluated. Both unheated rice bran (NRB) and rice brans heated at 60 °C for 15 min (HRB60) were used as disintegrant. The disintegrant properties of NRB and HRB60 were compared to sodium starch glycolate in a 500 mg paracetamol tablet formulate. The polyvinylpyrrolidone (PVP K30) was used as a binder with the wet granulation processes. Electron microscopy showed that NRB has a granular shape with smooth surface while HRB60 has a square shape with dented surface and also larger size. The swelling capacity of NRB and HRB60 in distilled water were 1.22 and 1.33 respectively. The Hausner's ratio of NRB and HRB60 were 1.24 and 1.19, respectively, indicating poor flow properties. The disintegration times of paracetamol tables containing 10% sodium starch glycolate, 10% NRB and 10% HRB60 as disintegrant were 100, 252 and 264 sec, respectively. These disintegration times are within recommended range of disintegration time for immediate release tablets (15 min or less). In conclusion, both unheated rice bran (NRB) and heated rice brans are promising disintegrant for immediate release tablets.

Keywords: rice bran, disintegrant, paracetamol

Catechol-bearing hyaluronic acid coated polyvinyl pyrrolidone/hydroxyl propyl-β-cyclodextrin/clotrimazole nanofibers for oral candidiasis treatment

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Abstract. This research aimed to develop clotrimazole (CT)-loaded mucoadhesive nanofiber patches for oral candidiasis. The three-layered sandwich-like nanofiber patches were prepared by electrospinning technique. The spinning solution for the middle layer composed of 8 %wt polyvinylpyrrolidone (PVP), 90 mM hydroxy propyl-β-cyclodextrin (HPβCD) and 10 % (wt to polymer) of CT in a solvent mixture of ethanol:water:benzyl alcohol. The outer layers were fabricated from a mixture of 1 %wt hyaluronic acid (HA) or catechol bearing hyaluronic acid (HA-cat) and 10 %wt polyvinyl alcohol (PVA) at varied weight ratios. The thickness of the outer layers was varied by adjusting the volume of coating polymer solution ranging from 1 to 3 mL. Desirable smooth nanosized fibers were obtained from the electrospinning process. Increasing the thickness of the outer layer brought about a significant increase in the fiber strength and flexibility. The viscosity of HA-cat/mucin mixture showed good polymer-mucin interaction indicating higher mucoadhesive property of the nanofibers. The drug loading capacity (LC) displayed the potential of the nanofibers for drug encapsulation. The highest LC value of 123.80 ± 5.61 μg/mg was obtained from the nanofibers coated with 1 mL of the coating solution. CT was rapidly released from the nanofiber in the first hour followed by a steady release. The released amount reach above 80% in 2 h. The nanofibers provided superior antifungal activity against *Candida albicans* compared to CT powder. Moreover, they were found to be nontoxic to the human gingival fibroblast cells. Thus, the sandwich nanofibers may be further developed to be a potential candidate for oral candidiasis treatment in the near future.

Keywords: mucoadhesive, nanofibers, hyaluronic acid, catechol, oral candidiasis, clotrimazole

The development of 3D-spheroid model for anticancer drug screening

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Abstract. One of the most important obstacles of anticancer drug development is an in vitro model for evaluating efficacy of the new anti-cancer candidates that may not correlate with in vivo and clinical results. Two-dimensional (2D) monolayer cultures are standard in vitro model for the current drug discovery research. However, they have failed to recapitulate the multicellular three-dimensional (3D) structure of tumours and their microenvironment. While, the differences in species between human and animal, and animal expenditure have limited in vivo study; the 3D – models may be the best alternative. Therefore, it is highly important to try to develop the 3D-models for drug discovery and screening with low budget. In this study, we developed 3D-models and demonstrated that 3D-models were better than 2D- models as they could mimic the tumour architecture and microenvironment. We utilized human liver cancer cells (HepG2), fibroblast cells and human umbilical vein endothelial cells (HUVEC) for creating 2D-mono-/co-culture models, 3D-co-culture models by hanging drop and ultra-low attachment (ULA) techniques. The models were evaluated by assessing shape, size, aggregation pattern, morphology, viability, albumin production and MRP2 expression. Our resulted revealed that the diameter of the 3D-monoculture spheroids was ranged between 140-458 micron, and the HepG2 exhibited the largest spheroids compared with other cell types. The 2D-monoculture model significantly produced albumin at the lower level than 3D-monoculture model ($p<0.05$, $n=6$). The developed 3D-monoculture model expressed MRP2 protein after 14 days, and the expression level is stable for 43 days. 3D-co-culture models of HepG2 and fibroblast expressed MRP2 protein after 7 days indicating 3D-models could recapitulate physiological condition of liver tumour tissue. Generating 3D models by hanging drop technique, spheroids were more symmetry, narrow size distribution, but low number of spheroids were obtained. ULA technique was quick and simple technique to generate high number of spheroids with a wide range of size distribution. We are further optimizing the ULA technique to achieve narrow size distribution of spheroids for cancer drug screening.

Keywords: spheroids, organoids, 2D-model, 3D-model, liver cancer, tumour

The development extemporaneous formulation for bitter taste masking of metronidazole suspension; a preliminary study

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Abstract. The conventional hospital formulation of extemporaneous metronidazole suspension has a bitter taste which is not favor in children, leading to untaking this drug. Therefore, the aim of this study was to develop formulation of extemporaneous metronidazole suspensions with a better taste masking, and physicochemical and microbiological stability. Sucralose was used as the main taste-masking agent in this study. There were 5 metronidazole suspension formulations with various concentrations of sucralose (1.5, 2, 2.5, 2.8 and 3.3% respectively). The bitter taste evaluation was performed in 40 -healthy volunteers with the age between 18-25 years old. The less bitter taste score formulations were tested for the stability in the light protected container at 4 and 25 Degree Celsius for 93 days. The analysis of stability was done at day 0, 7, 14 28, 56 and 93. The most favorite formulation from volunteers' ratting was contained 2.5% and 3.3% sucralose. According to the cost saving, the formulation with 2.5% sucralose was chosen to stability study. The physical stability study found that the preparation stored in light protected container at 4 and 25 Degree Celsius had the physical changed at day 56 and 28, respectively. The chemical stability study revealed that pH of the products was ranged from 4.25 to 4.34. The content of metronidazole was 95.96 $\pm$ 0.26 % and 96.6 $\pm$ 0.22 % of the labelled amount in 4 and 25 Degree Celsius at day 93, respectively. No microbial contamination was found during microbial assay study at day 93. In conclusion, the extemporaneous metronidazole suspension with sucralose 1.5 g (2.5%) had good favor and stable at 4 and 25 Degree Celsius (Physical stability at least 56 and 28 days, respectively and chemical stability at least 93 days).

Keywords: development, bitter taste masking, metronidazole suspension, extemporaneous

The application of lithium dose prediction equation from PK approach in children: case report from Yuwaprasart Waithayopathum Child Psychiatric Hospital

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Abstract. According to our previous retrospective research title "the Estimation of Pharmacokinetic parameters and accuracy of the predicted equation for lithium dose in children: a pilot study at the Yuwaprasart Waithayopathum Child Psychiatric hospital", resulted the estimation of Lithium dose equation from clearance value by modification from Yukawa's model; CL (mL/min) = [36.5+(0.242x BW (kg)-7.79]/ SCR (mg/dL) and lithium concentration (mg/l) = 4 x Dose (mg)/CL (mL/min) x73.89, respectively. This equation was fit in Thai children and gave less error. Therefore, we tried to apply this equation in the real case as prospective monitoring. The case was 14-year-old boy, weight = 48 kg, serum creatinine = 0.4 mg/dL, lithium concentration = 0.52 mg/L, Lithium dose was 900 mg /day (1 tablet at the morning and 2 tablets before bed). A patient presented side effect (polydipsia) of Lithium, although he responded to treatment. Using the above equation, 900 mg /day of Lithium, predicted its concentration in this patient equal to 0.48 mg/L. It was similar to actual concentration. Then, we decided to decrease the dose (2 tablets before bed only) to minimize side effect. The predicted lithium concentration by using this equation was 0.32 mg/L. This case represented the application of the predicted lithium dose equation by using a pharmacokinetic model in a clinical setting which may be able to generalize in routine work

Keywords, application, lithium dose, prediction equation, PK approach, children

Stability of mangiferin in lotion and its antioxidant activity

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Abstract. The antioxidant lotion containing 1% mangiferin, which was previously isolated from leaves of mango variety Nam Doc Mai (purity 93.15%, HPTLC analysis), was prepared. The lotion was an o/w type of emulsion with slightly yellow and coconut scent. The viscosity and pH of the lotion were 19,809 centipoise and 5.94, respectively. Stability studies were performed at room temperature (25±5˚C) with 70±10%RH and at high temperature (45±2˚C) with 75%RH. The amount of mangiferin in the lotion was investigated at various time intervals by HPTLC. The results showed that mangiferin in the lotion remained 80.30±1.87%LA after storage for 3 months at room temperature. While it remained less than 80%LA after storage for 2 weeks at high temperature and only 38.51±0.35%LA remained after 3 months of storage. An unknown degradant was detected at the R_f value of 0.24. It was produced increasingly when storage peroid taken longer, especially at the high temperature. UV spectra of the degradant and mangiferin suggested the similarity of their chemical structures. Antioxidant activity of the lotion was evaluated by DPPH radical scavenging method. Interestingly, the activity has not decreased at both temperatures during storage times even if mangiferin degraded. It was possible that the degradant could scavenge DPPH redical and chemical structure of the degradant might be xanthone.

Keywords: mangiferin, DPPH radical, HPTLC, antioxidant, c-glycosyl xanthone.

Solubility enhancement, solution and solid-state characterization of asiaticoside/sulfobutyl-ether-β-cyclodextrin

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Abstract. Asiaticoside (AS) is triterpenoid glycoside, which is one of the four major constituents of *Centella asiatica*. It has been attracted much for its significant wound healing activity but its application was limited due to its poor solubility. Hence, the aim of the study was to improve AS solubility via complexation with β-cyclodextrin (βCD) and its derivatives i.e., carboxy-methyl-β-cyclodextrin (CMβCD) and sulfobutyl-ether-β-cyclodextrins (SBEβCD). The complexation in solution-state was evaluated by phase-solubility technique and ^{1}H nuclear magnetic resonance (^{1}HNMR). The enhanced solubility was obtained with linear (A_L) type phase solubility profile with all CDs tested which represented 1:1 stoichiometry inclusion complex. SBEβCD exhibited the better complexation efficiency for AS than CMβCD and selected for further studies whereas βCD was excluded because of its limited aqueous solubility. The mode of inclusion was supported by ^{1}H-NMR and indicated that true inclusion complex was formed between AS and SBEβCD. The solid AS/SBEβCD complex was prepared by freeze-drying (FD) method and formation of complexation was determined by solid-state characterizations that were Fourier transform infra-red spectroscopy (FT-IR) and powder X-ray diffraction (PXRD) techniques. FT-IR showed the presence of interaction in the complex whereas PXRD revealed that binary complex was in amorphous state and/or presence of inclusion AS/SBEβCD complex. Thus, the formation of AS/SBEβCD complex could be beneficial to enhance the solubilization of AS.

Keywords: asiaticoside, cyclodextrin, solubility, characterization

Solubility enhancement of ibuprofen using solvent systems

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Abstract. Ibuprofen, a nonsteroidal anti-inflammatory drug, is practically insoluble in water. Poor aqueous solubility not only gives rise to variable oral bioavailability but also leads to determine rate of drug absorption. This study examined the solubility of ibuprofen using a series of pure solvents and the solvent mixtures prepared from water, ethanol, propylene glycol (PG), polyethylene glycol 400 (PEG 400), polyethylene glycol 600 (PEG 600), PEG-7 glyceryl cocoate (Cetiol® HE), caprylic/capric triglyceride (Myritol® 318) and PEG-40 hydrogenated castor oil (Kolliphor® RH 40) in order to achieve the concentration of ibuprofen up to 400 mg/mL. It was found that the solubility of ibuprofen was significantly improved by using ethanol, PEG-7 glyceryl cocoate, PEG 600, PEG 400 and PG with the value of 808 mg/mL, 450.31 mg/mL, 330.64 mg/mL, 323.39 mg/mL and 320.28 mg/mL respectively. As the result, a solubility increased with a decrease in the dielectric constant of the mixture, which corresponds to the system that has dielectric constant equal to 25. It was found that the co-solvent of PG: PEG 600 at the ratio of 6.54: 3.46 and PG: PEG 400: PEG 600 at the ratio of 6.47: 1.77: 1.77 improved the solubility of ibuprofen equal to 409.86 and 360.06 mg/mL respectively. Moreover, the PEG 600: water system with dielectric constant of 18.29 significantly improved solubility of ibuprofen as well (421.50 mg/mL). Thus, one of choices to design and develop liquid dosage forms containing ibuprofen 400 mg/mL, the drugs may require the system without a component of water like PG: PEG 600 system at the ratio of 6.54: 3.46 which gives the dielectric constant value equal to 25.

Keywords: Ibuprofen, co-solvent, dielectric constant, solubility

Selected colored medicinal Thai plants influence on antioxidant and acetylcholinesterase activities

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Abstract. Alzheimer's disease is a progressive neurodegenerative disorder, oxidative stress and acetylcholinesterase play a key role in the pathology. The aim of this study was to evaluate the influence of selected colored Thai plants on the key role of the neurodegenerative disorder, including the role of antioxidant defense and acetylcholinesterase inhibition. The hydrophilic extracts of *Clitoria ternatea* L. flowers (anchan, AC), *Hibiscus sabdariffa* L. flowers (krajeab, KJ), *Morus* alba L. fruits (mhon, M) and *Oryza sativa* L. seed (rice berry, RB) were extracted using 15% of 0.1 N HCl in ethanol and dried through vacuum oven at 50°C. The superoxide radical scavenging and modified Ellman's method were conducted for antioxidant and acetylcholinesterase activity, respectively. The superoxide radical scavenging activity of RB extracts was superior, followed by KJ, AC and M extracts. Similarly, the acetylcholinesterase inhibition activity of RB extracts was superior, followed by AC, M and KJ extracts. The hydrophilic extracts were represented the phytochemicals to play role of antioxidant and acetylcholinesterase inhibition. The results suggested that the hydrophilic rice berry extracts showed anti-cholinesterase activities which may prevent Alzheimer's disease.

Keywords: alzheimer's, acetylcholinesterase, antioxidants, medicinal plant

Purple rice and yeast β-glucan extracts influence on liver oxidative stress associated-colitis induction in wistar rats

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Abstract. The oxidative stress is the key to the disorders in many organs. Especially, in the liver is the important center for biomolecular generation. The aim of this study was to evaluate the influence of phytochemicals in purple rice and yeast to preserve liver from oxidative stress on colitis induction model. The five groups of animals such as G1 (control), G2 (inducted by 3% dextran sodium sulfate in drinking water ad libitum), G3 (induced+500 mg/kg body weight (bw.) purple rice extracts (p.o.) daily), G4 (induced+250 mg/kg bw. β-glucan extracts (p.o.) daily) and G5 (induced+500 mg/kg bw. purple rice & 250 mg/kg bw. β-glucan extracts (p.o.) daily) were investigated. G1 to G5 were sacrificed at 14 days to evaluate malondialdehyde (MDA) and glutathione peroxidase (GPx) in liver. The liver tissue was homogenized to release the MDA and GPx for evaluation by colorimetric methods. The results found that the colitis-induced rats (G2) were lost ~17%, also MDA level was increased to 75% while GPx was decreased to 57%. Interestingly, the combinational intervention showed superlative influence. The level of MDA in colitis induction was decreased significantly at 28.80, 27.54 and 21.24% in G5, G3, and G4, respectively. Besides, the level of GPx in colitis induction was increased significantly at 107.34, 59.44 and 49.47% in G5, G3, and G4, respectively. The results suggested that the purple rice and/or yeast β-glucan extracts exhibited antioxidant activity that prevent the liver damage from colitis.

Keywords: colitis, oxidative stress, purple rice, β-glucan

Production and structural characterization of polysaccharides from marine actinomycetes

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Abstract. Microbial polysaccharides are biopolymers produced from different microorganisms. Due to their interesting bioactivities with low toxicity, microbial polysaccharides have been widely applied in pharmaceutical and cosmetics industries. Nowadays, exploring new microbial strains for better polysaccharide producers have been increasing continuously. Herein, we investigated the ability of different actinomycetes screened from different mangrove areas in Thailand for polysaccharide production, in particular fructan. These isolates were initially grown in ISP2 (International Streptomyces Project2) media containing 20% (w/v) sucrose at 30 ^{o}C for seven days. The polysaccharides in the supernatant were then precipitated by adding two volumes of cold absolute ethanol. The types and components of polysaccharide were analyzed by acid hydrolysis and thin layer chromatography. We found that one of the isolates, PL-4-6, was able to produce fructan. After structural characterization by FT-IR and NMR techniques, the fructan from PL-4-6 had a similar structure to levan. Without an optimization of the culture condition, the obtained yield of levan from PL-4-6 was 2 g.L^{-1}. Taken together, this study suggested that PL-4-6 might be used as another microbial source of levan production for pharmaceutical applications in the future.

Keywords: microbial polysaccharides, actinomycetes, fructan, levan

Development of value added organic rice for commercialization: food and cosmetic products

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Abstract. Thailand is well known for the last 3 decades as the world's largest rice exporter. Therefore, rice production has played an important role in the economic and social development of the nation. However, the rice-farmers are typically faced with several problems about the uncertainties of their crop. The drought in summer with insufficient rain destroyed a great amount of rice. Nevertheless, the excessive rain could also do the same. This problem significantly affects the price of Thai rice. Open learning developing local-level of knowledge in connection with 'research' may overcome the farmer's solution. The aim of this study was to develop value added organic rice for commercialization. Rice procured from Ubon Ratchathani province was used in this study. The chemical compositions of rice bran, defatted rice bran and rice bran protein from different sources were studied in order to apply for food and pharmaceutical products. Organic rice bran oil (O-RBO) encapsulated beads, organic rice bran (O-RB)-facial cleansing scrub and O-RBO loaded nanovesicles were designed and developed. The finding indicated that the by-product from organic rice can be used as a potential protein source for food and pharmaceutical product. Moreover, the encapsulated O-RBO beads and O-RB facial cleansing scrub and O-RBO loaded nanovesicles can be used as potential ingredients for cosmetic industries.

Keywords: rice bran oil, oryzanol, facial cleansing, scrub, bead, nanocosmetic

Fabrication of enteric release tablet without coating process by using bleached shellac

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Abstract: The purpose of this study was to design an enteric release tablet by a simple method of tableting and avoiding the coating process. Polymers which had a protection ability against acid were required for tablet formulation. Bleached shellac was selected as an excipient due to colorless property, a protection ability against acid in upper gastrointestinal tract and release ability in lower gastrointestinal tract. Bleached shellacs in salt and acid forms were dissolved in 12 ml of 95% ethanol and used as a binding agent to get a concentration of 5, 7.5, 10, 12.5, 15, 17.5 and 20 %w/w of total formulation. 60% lactose, 30% Avicel pH 102 and 10% paracetamol (model drug) were mixed and tablets were prepared by wet granulation method with the weight of 550 mg and hardness of 8-10 kg. The tablets were evaluated for their disintegration and drug release at pH 1.2 and 6.8 and kept at 40 ºC, 75% RH for 6 months. The findings showed that the disintegration and amount of release were dependent on type and concentration of bleached shellac. Only the bleached shellac in salt form could protect the release of drug at pH 1.2 for 2 h and could release completely at pH 6.8 but not in acid form. After 6 months of storage, 15% bleached shellac in salt form could still protect against acid and complete release at pH 6.8 for 4 h. Although the bleached shellac showed protection ability against acid, it could not comply with the required criteria of enteric release tablet. Further study is hence required. However, the stability test of tablets prepared with bleached shellacs in salt form could show protection against acid and complete release at pH 6.8 after 6 months of storage. Bleached shellacs in salt form as a binding agent showed a good approach for the fabrication of enteric release tablets without coating process by using a proper concentration of bleached shellac. Therefore, the attempt to design an enteric tablet by a simple method and avoiding the coating process is achieved.

Keywords: bleached shellac in salt form, enteric tablet, coating, wet granulation

Influence of process parameters on the characteristics of hydrophilic drug-loaded microparticles through double emulsion solvent evaporation technique

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Abstract. The purpose of this study was to investigate the influence of process parameters on the characteristics of microparticles using double emulsion solvent evaporation method for encapsulation of hydrophilic drug. Donepezil hydrochloride (DPH), a reversible cholinesterase inhibitor, was selected as a model hydrophilic drug. Prior to conducting an experiment, the target particle size of microparticles was set at approximately 200 μm. The investigated process parameters include pH of outer water phase, stirring time, polymer amount, and volume of outer water phase. The results showed that DPH-loaded microparticles was successfully prepared in two steps. In the first step, the primary emulsion was prepared by dissolving DPH in distilled water before emulsifying in dichloromethane (DCM) containing different amounts of poly(butylmethacrylate-co-2-dimethylaminoethyl-methacrylate-co-methyl-methacrylate) (PBM-DM-MM) using ultrasonic probe. In the second step, the primary emulsion was emulsified in polyvinyl alcohol (PVA) solution by overhead stirrer to prepare double emulsion. After solvent evaporation, the microparticles were collected by centrifugation and washed with distilled water. Based on the statistical analysis, stirring time, polymer amount and volume of outer water phase were the main significant parameters influencing particle size of microparticles.

Keywords: double emulsion, encapsulation, donepezil hydrochloride

Development of nanogels using gamma radiation induced crosslinking of inter-polymer complexes

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Abstract. Nanogels is a dosage form that can deliver a drug to a specific target. Optimal choices of polymers are good beginning to prepare nanogels. The objective of this study was to select appropriate polymers for preparing nanogels by radiation-induced crosslinking. The inter-polymer complexes (IPCs) of chitosan with polyvinyl pyrrolidone (CS-PVP), sodium hyaluronic acid with polyvinyl pyrrolidone (HA-PVP) and chitosan with sodium hyaluronic acid (CS-HA) were chosen to prepare nanogels using gamma radiation-induced crosslinking. IPCs were irradiated at 1 kGy of the different polymer concentrations (0.10 - 0.50 mg/ml). The particle size was measured by a zetasizer. Stability of the nanogels was taken into consideration. Only CS-HA IPCs could form suitable nanogels with the particles size of approximately 150 nm. Increasing CS and HA concentrations resulted in the increase in size of the particles. CS-HA nanogels was stable up to 7 days in the aspects of particles size, polydispersity index, pH and zeta potential. It is possible to load a drug to the CS-HA nanogels used as a delivery system. However, a further drug loading and release study are required.

Keywords: nanogels, gamma radiation, chitosan, hyaluronic acid, polyvinyl pyrrolidone.

Development of transdermal patch from *Centella asiatica* crude extract

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Abstract. *Centella asiatica* has been widely used for treatment of scar. The duration of action can be prolonged by preparing the *Centella asiatica* crude extract in the form of transdermal patchs. The objective of the study was to select suitable polymers and sources of crude extract for preparing transdermal patches containing *Centella asiatica* crude extract and to study the effect of extract on mechanical and bioadhesive properties of the transdermal patches. The active compound, asiaticosides, was analyzed using HPTLC. The quantitative determination of asiaticosides from three companies found that the extract of *C. asiatica* from two companies contained asiaticosides. The transdermal patches containing 5% by weight of chosen *C. asiatica* extract were prepared by solvent casting method using Eudragit RS, RL and the blend of Eudragit RS and RL in the ratio of 1:1. The trandermal patches were evaluated for mechanical, bioadhesive properties and extract release. The results from tensile strength showed that the transdermal patches containing *C. asiatica* extract had higher strength and Young's Modulus than the film without *C. asiatica* extract. The elongation of transdermal patches decreased with the addition of *C. asiatica* extract. It was similar to mechanical properties. The bioadhesive property of patches would be decreased with the addition of *C. asiatica* extract. After adding 5% by weight of *C. asiatica* extract, the mechanical and bioadhesive properties of transdermal patches prepared by Eudragit RL and the polymer blend would be better than the patched prepared by Eudragit RS. However, the release of extract from the patches prepared by Eudragit RS would be faster than those prepared by Eudragit RL and polymer blend. In conclusion, *C. asiatica* extract had an impact on mechanical and bioadhesive properties of transdermal patches prepared by Eudragit RS, RL and their blend. Eudragit RL and the polymer blend were chosen for further development of transdermal patches containing 5% by weight of *C. asiatica* extract due to the mechanical and bioadhesive properties with improvement of extract release.

Keywords: *Centella asiatica* extract, asiaticoside, transdermal patch, eudragit RS, eudragit RL

Physicochemical properties of rice flour at different ripening stages as potential excipients for food and pharmaceutical products

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Abstract. The physiochemical properties of rice four were determined using a high amylopectin rice comparing between two stages: dough and mature stages as aimed to apply the flour for suitable food or pharmaceutical product development. Proximate analysis, amylose content, total starch content and thermal properties of flour were conducted with triplication. It was found that most of chemical components did not different, exception of lipid content. However, the pasting properties of mature ripening flour provided a harder texture than the dough stage flour despite of the swollen starch granule was found less than the dough stage. It could be suggested that some complex chemical interaction may occur during heating and cooling of dough stage flour.

Keywords: rice flour, ripening stages, physicochemical properties

Formulation and evaluation of ginger lozenges

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Abstract. The aim of this study was to formulate herbal lozenges containing ginger extract to presented as an alternative oral dosage form to be used as an effective and safe treatment for nausea and vomiting. The lozenges were formulated by molding method and wet granulation method for producing candy lozenges and compressed lozenges containing ginger extract, respectively. Ginger rhizomes were extracted with 95% ethanol by simple maceration process. The extract was identified by using high performance liquid chromatography (HPLC) method to analyze for 6-gingerol, which is the major chemical composition that act as the active compound. The physical properties of formulated lozenges such as appearance, hardness, friability and organoleptic properties were carried out for the optimization of the formulation. The result show that 6-gingerol content in the ginger extract that used in this study was found to be 12.59% w/w. Ginger extract can be formulated into lozenges. The formulation of candy lozenges (3.4 g per lozenge) containing 13.5 mg of ginger extract with liquid glucose, sucrose, citric acid, propylene glycol and the formulation of compressed lozenges (500 mg per tablet) containing 6.75 mg of ginger extract with sugar free based excipients as mannitol, corn starch, starch gel, sodium stearyl fumarate, aspartame and citric acid were the optimized formulations that successfully formulated and evaluated.

Keywords: ginger, molded lozenges, compressed lozenges, wet granulation

Formulation and *in-vitro* evaluation of fast dissolving tablets using superdisintegrant blend with effervescent material

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Abstract. The objective of this study was to formulate fast dissolving tablets (FDTs) using superdisintegrant and effervescent material. The tablets were prepared by direct compression method. The effect of superdisintegrant content was studied. The weight and hardness were controlled within the range of 500±20 mg and 50±10 N, respectively. Tableting properties including weight, thickness, diameter, friability, hardness, wetting time, water absorption ratio and *in-vitro* dispersion time were evaluated. As a result, the physical properties of tablets were within the required limit. As increasing the amount of sodium starch glycolate, the water absorption ratio had a tendency to increase. However, the wetting and dispersion time took more longer. By effect of adding effervescent material (tartaric acid and sodium bicarbonate), the wetting and dispersion time were lower. The time showed less than 3 min that represented a good characteristic of FDTs. This study showed that, among the designed formulations, the formulation containing effervescent material emerges as the overall best formulation based on drug dissolving characteristics.

Keywords: fast dissolving tablets, direct compression, superdisintegrants, effervescent

Chemical constituents and antioxidant activities of *Curcuma roscoeana* Wall. rhizomes

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Abstract. The hydrodistillated essential oil and crude ethanolic extract from the fresh rhizomes of *Curcuma roscoeana* were investigated for their chemical constituents and antioxidant activities. The major component of the essential oil was found to be tricyclene (45.18%). Preliminary phytochemical screening results of the crude ethanolic extract revealed the presence of triterpenoids. Antioxidant activities were evaluated using five different methods including DPPH radical scavenging, hydroxyl radical scavenging, superoxide anion radical scavenging, ferrous ion chelating and ferric reducing power assays. *L*-ascorbic acid and ethylenediaminetetraacetic acid (EDTA) were used as a positive control. The essential oil and crude ethanolic extract showed strong hydroxyl radical scavenging and superoxide anion radical scavenging activities as well as high ferric reducing power.

Keywords: *Curcuma roscoeana*, antioxidant activity, Zingiberaceae

Screening of the content of polyamines in bird pepper by TLC and HPLC methods

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Abstract. The abundant biologically active polyamines, putrescine (PUT), spermidine (SPD), and spermine (SPM), are found in almost every organism. These biogenic amines are important for cell growth and viability. Intracellular polyamines are supplied by biosynthesis and by the intake from diet. Polyamines have been gaining much attention due to their effects on health and diseases, especially in aging pathology. Unfortunately, the available polyamine contents data in foods are still insufficient. Normally, high-performance liquid chromatography (HPLC) is the most used technique for polyamine determination but HPLC-based methods are expensive and time-consuming. In the present study, the determination of polyamine contents in Bird pepper or Phrik-khi-nu (*Capsicum annuum* L.), frequently used ingredient in Thai cuisine, was attempted by TLC-densitometry and TLC–image analysis as well as HPLC. The amount of PUT, SPD, and SPM in Bird pepper analyzed by TLC and HPLC were in a comparable concentration range approximately at 300-405 nmol/g of fresh weight (FW). Bird pepper exhibited to be rich in SPM, PUT and SPM, respectively.

Keywords: polyamine, TLC, image analysis, food

Heavy metals in Thai *Arachis Hypogae* L. determined by inductively coupled plasma mass spectrometry (ICP-MS)

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Abstract. The contamination of heavy metals in food is primary health concern nowadays. The consumption of heavy metals adulterated food has related to the several serious illnesses. The aim of this study was to determine Ni, As, Cd, Ba and Pb levels in edible peanuts. Raw peanut kernels were digested with nitric acid by Milestone microwave digestion system. The digested solution was diluted and injected on inductively coupled plasma mass spectrometry (ICP-MS). The concentrations of each heavy metal were calculated against multi-element standard solution calibration curves. The results found that the quantity of Ni and As was not significantly different among peanut varieties; Thainan-IV, Kalasin-I and Kalasin-II. Cd and Ba were found significantly high amount in Kalasin-I and Kalasin-II respectively while Pb was detected only in Kalasin-II variety at low level. Overall, heavy metals found in this study were in the limit of contaminated food allowed by the ministry of public health, Thailand.

Keywords: heavy metals, ICP-MS, peanut

Honokiol and magnolol induced DAMPs releases mediated apoptosis induction on human cholangiocarcinoma cells

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Abstract. Introduction and Objectives; Cholangiocarcinoma (CCA) is biliary tract malignancy. Because no specific biomarkers are available, CCA patients frequently present with disseminated tumour that is too late for curative treatment, leading to high mortality rate. Honokiol and magnolol are the hydroxylated biphenyl compounds isolated from *Magnolia officinalis*. Many studies have reported that honokiol and magnolol effectively induced cancer cell death. However, the evidence of the effects of these compounds on CCA has not yet been reported. In this study we aim to study the effect of honokiol and magnolol on human CCA cells. Together with the induction effect of damage-associated molecular patterns expression of these compounds, which is the danger signal for immune system activation. The anti-tumour activity of honokiol and magnolol was determined in context of the cytotoxicity effect, anti-proliferation effect and the mechanism on the induction of cell apoptosis. These compounds exhibited the cytotoxicity on human CCA cells KKU-100 and KKU-213L5. The underlying mechanism was confirmed by Annexin V/PI staining and caspase-3 expression. The results indicated that honokiol and magnolol induce CCA cell death mediated by apoptosis mechanism. Moreover, the effect of honokiol on DAMPs expression from CCA cells also investigated. Interestingly that induction of cell apoptosis by honokiol cause of damage-associated molecular patterns (DAMPs) release including high mobility group box 1 (HMGB1) and heat shock proteins 90 (HSP90). Therefore, in this study we demonstrated that honokiol and magnolol have potential anticancer properties that are promising compounds for alternative CCA treatment. Especially, the induction of DAMPs expression which is fascinated to combine as immunoadjuvant of cancer immunotherapy for CCA treatment.

Keywords: honokiol, magnolol, cholangiocarcinoma, DAMPs

Polymethacrylates as polymeric film formation in patches containing α-mangostin and resveratrol

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Abstract. Polymethacrylates polymeric film formation in patches containing α-mangostin and resveratrol were developed using solvent casting method. Eudragit® E100 (E) and Eudragit® L100 (L) were dissolved in ethanol and the plasticizer (propylene glycol (PG) and polyethylene glycol (PEG) 400) was individual added and followed with the drying process. The dried films were evaluated for the morphology and flexibility. After the stable film was achieved, the α-mangostin and resveratrol were incorporated into the film. The variation of weight and thickness, swelling property, pH surface, mechanical properties and drug content of patches was evaluated. Fourier transform infrared spectrophotometry (FT-IR) was also conducted to confirm that drugs were qualitatively loaded into the patches. The results indicated that patch of L and PG was found to be stable. PG enhanced the flexibility of patch. The patches were less variation in weight and thickness. This patch did not effect to the physiological pH in the human body. In addition, patch had a tensile strength high enough to withstand tearing during handing. The qualitative and quantitative analysis indicated the α-mangostin and resveratrol was well incorporated in this patch. These results suggest that polymethacrylate polymer could be a promising polymeric film formation in patches for drug delivery.

Keywords: polymethacrylates, polymer, film, α-mangostin, resveratrol

Physical stability of astaxanthin from *Haematococcus pluvialis* loaded in micromeulsion as a cosmetic ingredient for melanogenesis inhibition

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Abstract. In this study, astaxanthin (ASTA), with potential anti-tyrosinase and anti-melanin synthesis in melanoma cells ($B_{16}F_{10}$) was developed as a cosmetic ingredient in the form of microemulsions (MEs). The results showed that ASTA (1 mg/mL) had no toxic effects on melanoma cells and it exhibited high potential for reduction of tyrosinase and melanin content, representing 80.57% and 75.86%, respectively. However, the use of ASTA is limited due to its low stability resulting from its decomposition under light, heat, and oxygen. In order to overcome this drawback, ASTA was encapsulated within ME. ASTA-MEs, consisting of 0.5% w/w of ASTA, oil, surfactant and water, were prepared using titration method. The effect of IPM concentration into microemulsions were investigated at 10 % w/w (ASTA-ME1) and 20% w/w (ASTA-ME2). The physical stability after accelerated condition of all the formulations was also investigated. The results indicated that a thermodynamically stable of microemulsion could improve the physical stability of ASTA. Nonetheless, the oil concentration had a slight influence on the physical stability of ASTA-ME1 and ASTA-ME2. In conclusion, nanoencapsulation can improve the physical stability of pigment extract to be used as a cosmetic ingredient in skin brightening products.

Keywords: stability, melanogenesis induction, astaxanthin, microemulsions

PEGylated plier-like cationic niosomes on gene delivery in HeLa cells

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Abstract. Lipid-based formulations have been used as a widespread carrier to improve gene delivery. Niosomes, one type of lipid-based vesicular systems are produced from non-ionic surfactants which are generally inexpensive and potentially more stable than phospholipids. This article was to develop PEGylated cationic niosomes for DNA delivery. Thin film hydration and sonication method were applied for cationic niosomes. The niosome formulations were composed of Span 20, cholesterol (Chol) and plier-like cationic lipid B (PCL-B) with or without cholesterol-polyethylene glycol 2000 (Chol-PEG). The physicochemical properties of cationic niosomes and nioplexes were evaluated including particle size, zeta potential, DNA condensation and serum protection. The transfection efficiency and cell viability were examined in HeLa cells. The particle size and surface charge of PEGylated cationic niosome containing Span 20: Chol: PCL-B: Chol-PEG at the molar ratio of 2.5: 2.5: 1.5: 0.14 (N-PEG2) were 129.47 ± 2.15 nm and 25.93 ± 4.18 mV, respectively. These PEGylated cationic niosomes could condense pDNA into the nanosize particles and also enhance the serum protection ability for at least 6 h. Moreover, N-PEG2 exhibited high transfection efficiency in comparison with lipofectamine® 2000 and low cytotoxicity. Therefore, the novel PEGylated cationic niosomes have the capability to develop as a promising potential carrier for DNA delivery.

Keywords: cholesterol-PEG, PEGylated niosomes, gene delivery, plier-like cationic lipids

Optimization of *Boesenbergia rotunda* extract-loaded polyvinyl alcohol hydrogel wound dressing by Box-Behnken design

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Abstract. The objective of this study was to optimize fabrication variables that affected desirable properties of dressings. *Boesenbergia rotunda* extract incorporated PVA hydrogels for wound dressings were fabricated by freeze-thaw method. The fabrication variables including PVA concentration (15, 17.5 and 20 % w/w), freeze-thaw cycle (2, 3 and 4 cycles) and extract loading (30, 40 and 50 % w/w) were studied and optimized. Effects of variables on the hydrogel wound dressing properties were determined by using Box-Behnken design and response surface method. Hydrogel properties such as tensile strength, elongation at break, Young's Modulus, water content, swelling and erosion were measured and used as the designed responses. From statistical data analysis ($p < 0.05$), the polynomial quadratic equation which indicated the significant effects of fabrication variables on the hydrogel properties was generated. In conclusion, desirable *B. rotunda* extract loaded PVA hydrogel dressing was favorably designed. The optimized PVA concentration, freeze-thaw cycle and extract loading were 17.07 % w/w, 3.86 cycles and 50 % w/w, respectively.

Keywords: hydrogel dressing, freeze-thaw, polyvinyl alcohol, Box-Behnken design

Niosomes containing spermine-based cationic lipid with different linkers for siRNA delivery

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Abstract. Niosomes are a lipid nanoparticle which have been widely used as non-viral carrier for therapeutic DNA or siRNA. They are formulated from non-ionic surfactant and other helper lipids. The aim of this study were to formulate niosome containing spermine-based cationic lipid with different linkers and to evaluate the efficiency of siRNA delivery in cervical cancer cell (HeLa cell). The niosomes were formulated from cholesterol (Chol), Span 20 and different cationic lipid (Ay, By, Cy and Dy) at various molar ratios. The properties of niosomes and ability of niosome to complex with siRNA were characterized. The cellular uptake, gene silencing efficiency and cytotoxicity were also determined. From the results, niosomes formulated at Chol: Span20: lipid molar ratio of 2.5:2.5:2 showed positive zeta potential and they were in nanosize (<200 nm). The binding ability of cationic niosomes to siRNA depended on types of cationic lipid. Among niosome/siRNA complexes, the niosome By/siRNA complex provided the highest gene silencing efficiency at weight ratio of 20. The highest cellular uptake also obtained by using niosome By as a carrier. The cytotoxicity revealed that cationic niosomes had low toxicity (cell viability > 80%). In conclusion, the cationic niosomes prepared from Chol, Span 20 and spermine-based cationic lipids are able to complex with siRNA and suitable for siRNA delivery with low toxicity.

Keywords: spermine-base cationic lipid, niosome, siRNA delivery

Natural furanocoumarin as potential oral absorption enhancer

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Abstract. Bioavailability of orally administered drugs can be influenced by many factors. Poor drug absorption across the intestinal membrane is one of the factors that contribute to low bioavailability of drugs. It has been suggested that the metabolism/active efflux in the small intestine is involved in the poor absorption of many drugs. Intestinal CYP3A4 and P-gp work coordinately to reduce the intracellular concentration of drugs. Recently, bioenhancers have been identified and extensively studied. The aim of this study was to evaluate natural furanocoumarins found in juices of common lime and kaffir lime as the potential enhancers for oral delivery by means of modulating CYP3A4 and/or P-gp activities. The role of isolated furanocoumarins on CYP3A4 was assessed by testosterone 6β-hydroxylation reaction, while the effect on P-gp was investigated using R123 and CAM uptake studies in Caco-2, LLC-PK_1 and LLC-GA5-Col300. In the present study, we demonstrated that isopimpinellin isolated from common lime is the best CYP3A4 inhibitor among 4 isolated furanocoumarins. The result indicates that isopimpinellin would possibly act as a bioenhancer by inhibiting pre-systemic metabolism. 6′,7′-dihydroxybergamottin found in kaffir lime is a dual inhibitor of CYP3A4 and P-gp. This finding suggests that 6′,7′-dihydroxybergamottin could potentially be used as a bioenhancer by inhibiting pre-systemic metabolism and efflux mechanism. However, *in vivo* study should be further conducted to confirm these effects in the body.

Keywords: bioenhancer, CYP3A4, 6′,7′-dihydroxybergamottin, efflux, furanocoumarin, isopimpinellin, inhibition, metabolism, oral absorption enhancer, P-gp

Stability of mulberry extract in skin cosmeceutical formulation on their flavonoid content and biological activities under extreme conditions

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Abstract. The cosmeceuticals formulations have to check for the bioactivity and safety. The stability of bioactive content and biological activities are considered as important facror for cosmetics. The aim of this study was to evaluate the stability of phytochemicals and biological activities of mulberry extract-loaded cream formulation to suggest the appropriate condition for storage. The hydrophilic mulberry extract was loaded in the oil-in-water cream at 0.1%w/w. Then, this formulation was stored at different storage conditions (4ºC, 45ºC and UV-light at 12/12 hr. of dark/light) for 90 days. Total flavonoid content was determined through quercetin equivalent (QE). The biological activities such as anti-oxidant and anti-tyrosinase were evaluated through trolox equivalent antioxidant capacity (TEAC) and kojic acid equivalent (KE), respectively. The results showed that the initial value at day 0 of flavonoid content, anti-oxidant and anti-tyrosinase activities were 246.59±12.13 QE µg/g cream, 0.55±0.03 TEAC mg/g cream and 3.02±0.02 KE ng/g cream, respectively. The storage at 4°C condition nicely represented the highest bioactive content and biological activities, followed by light and stored at 45ºC. Additionally, after 90 days of storage at 4ºC, 45ºC and light conditions, the flavonoids content was degraded by 4.96, 29.13 and 21.61%, respectively. Likewise, the same condition series presented the degradation of TEAC values at 2.57, 24.27 and 17.34%, respectively. Moreover, the degradation of KE values at 5.30, 31.13 and 22.52% were observed, respectively. The results suggested that the storage at 4ºC helps to maintain the bioactive content and biological activities of hydrophilic mulberry extract in a cream formulation.

Keywords: cosmeceuticals, stability, mulberry, flavonoids

Molecular docking study of anthocyanidins and anthocyanins as acetylcholinesterase inhibitors

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Abstract. Anthocyanidins and anthocyanins are flavonoid derivatives as known as plant-derived color pigments in many red, purple and blue fruits and vegetables. Anthocyanins are in the form of glycosides, while anthocyanidins are aglycones. Many experiments indicated various health-benefits of these phytochemicals, especially neuroprotective effect *via* acetylcholinesterase (AChE) inhibition. To understand the binding interactions of some anthocyanidins and anthocyanins to the active site of AChE and to predict *in silico* inhibitory activity, molecular docking study was performed using AutoDock 4.2. Docking results showed that pelargonidin-3-glucoside exhibited the best docking profile in terms of good binding affinity and inhibitory activity against human AChE. The binding mode of pelargonidin-3-glucoside was comparable to donepezil, but different to other anthocyanins. The presence of glucose moiety in pelargonidin-3-glucoside structure seemed to play a crucial role for an additional binding interaction nearby the catalytic site (CS) of enzyme, however, enzyme-labile and high polar properties of this functionality may diminish the ability of the compound as a potential inhibitor against AChE.

Keywords: anthocyanidins, anthocyanins, acetylcholinesterase inhibitor, molecular docking

Mechanical properties of pectin/eudragit blend films

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Abstract. The present work aimed to prepare the pectin film using the different types of Eudragit® as a polymer blends and glycerine as a plasticizer and to study the mechanical properties of the films. The mixing of polymer mixture was carried out using distilled water as a solvent throughout the experiment. The polymer mixture was transferred to Petri dish and evaporated the solvent in hot air oven. The maximum positive force and percentage of elongation at break were found the range of 63.58-409.94 g and 74.92-145.42%, respectively for pectin film-based Eudragit® RL 30D polymer blends, 87.12-409.94 g and 74.42-145.42%, respectively for pectin film-based Eudragit® RS 30D polymer blends, 76.50-409.94 g and 72.68-145.42%, respectively for pectin film-based Eudragit® NM 30D polymer blends, and 137.12-409.94 g and 44.42-145.42%, respectively for pectin film-based Eudragit® NE 40D polymer blends. The results indicated that the mechanical properties of the pectin film decreased with inclusion of various types of Eudragit® ($p < 0.05$). However, it was found that the pectin film-based different types of Eudragit® polymer blends have high potential to be used in pharmaceutical applications. Further investigation related to the incorporation of drugs or herbal extracts and the *in vitro* evaluation are recommended.

Keywords: Pectin; Eudragit®, mechanical properties, film, polymer blends

Matrix forming behavior of doxycycline hyclate-loaded beta-cyclodextrin *in situ* forming matrix and microparticle

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Abstract. *In situ* forming microparticle (ISM) is an injectable emulsion drug delivery system comprising drug loaded in polymeric solution as internal phase and oil combined with emulsifier as external phase. This emulsion transforms into microparticle after contact an aqueous environment by solvent exchange mechanism. This study developed ISM using internal phase comprising 5% w/w doxycycline hyclate (DH) loaded with various concentrations of beta-cyclodextrin (β-CD) using *N*-methyl-2-pyrrolidone (NMP) as a solvent and external phase containing olive oil combined with glyceryl monostearate (GMS). High concentrated β-CD solutions in NMP are used as the internal phase of ISM. Matrix forming behavior is evaluated for their particle size, transformation to microparticle, pH, rate of matrix formation, contact angle and surface tension. Each emulsion had similar pH values about 3.5-4.1 and transformed into microparticles (particle size about 60 µm) after contact with phosphate buffer pH 6.8. The particle sizes of each preparation decreased significantly after transformed into microparticles and the more microparticles were evident with time. The rate of matrix formation of ISM was apparent slower than its internal phase and was slower with time. Contact angle of ISM and its internal phase showed good wetting which the surface tension of 35% w/w β-CD ISM was 44.19 mN/m. The β-CD ISM exhibited as the potential delivery system for incorporation of active compounds.

Keywords: *in situ* forming matrix; *in situ* microparticles; doxycycline hyclate; beta-cyclodextrin

Formulation of chitosan-ethylenediaminetetraacetic acid/poloxamer gel containing fruit's hull of *Garcinia mangostana* extract

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Abstract. The purpose of this study was to develop the formulation of chitosan-ethylenediaminetetraacetic acid (EDTA)/poloxamer containing *Garcinia mangostana* (GM) extract gel for oral cavity. The GM extract with a concentration of 0.5% w/w was incorporated into a gel formulation. The physical appearance, pH, viscosity and percentage label amount of GM extract gel were performed. The in vitro antioxidant activity of gel were evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. The antibacterial activity against Staphylococcus aureus was evaluated by the zone of inhibition method. The mucoadhesive property was investigated using viscosity technique. The results illustrate that the chitosan-EDTA/poloxamer containing GM extract gel had a yellow colour of GM extract. The pH of a gel was in the range of 4.47 – 6.87. The percentage label amount of gel was in the range of 98.71 – 99.37% and the viscosity of gel were in the range of 9607 – 14784 mPa/s. The Sol-Gel temperature ($T_{Sol.Gel}$) was 35 ºC. All gel exhibited antioxidant activity which evaluated by DPPH method. The zone of inhibition of gel against S.aureus was in the range of 8.17 – 10.52 mm. The chitosan-EDTA may improve the mucoadhesive property of gel. In conclusion, the chitosan-EDTA/poloxamer containing GM extract gel may have the potential for pharmaceutical application.

Keywords: chitosan-ethylenediaminetetraacetic acid, poloxamer, *Garcinia mangostana* extract

Formulation and evaluation of spironolactone anti acne gels

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Abstract. The objective of this study was to find appropriate compositions to formulate 5% spironolactone topical gel with good stability and physical characteristics for the treatment of hormonal acne. The 5% spironolactone gels were prepared using hydroxypropyl methylcellulose as the gelling agent. The effects of additives (tea tree oil, lactic acid and PEG 400) on the physical characteristics such as clarity, color, pH, viscosity and % drug content, had been investigated after preparing and storing for 1 month at room temperature and under accelerated condition (45°C/75%RH). After preparation, the gels were clear, colorless or yellowish in color in some formulas due to the adding of lactic acid. The pH was in between 3.69 - 5.76, the viscosity was in between 311.6 ± 4.2 to 676.4 ± 7.5 centipoises and the drug content was 89.06% to 95.87%. The formulas containing PEG 400 were found to be higher in the viscosity compared to those without PEG 400. After being stored for 1 month, the formulations containing tea tree oil were found to have less mercaptan-like odor of the drug. The lactic acid containing formulation produced more unacceptable odor. After storage at room temperature and under accelerated condition for 1 month, the assay value decreased from the initial time from 2.2% to 17.7% and 3.3% to 23.9%, respectively. It was found that, tea tree oil can be used as a good odor masking while lactic acid should be avoided. The addition of PEG 400 provided higher viscosity and had no advantage on drug stability.

Keywords: Spironolactone, acne gel, antiandrogen, hydroxypropyl methylcellulose

Development of microemulsions containing *Carthamus tinctorius* extract for 5α-reductase inhibition

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Abstract. The purpose of this study was to develop micromeulsion consisting of *Carthamus tinctorius* floret extract (CT) as an ingredient to inhibit 5α-reductase activity. CT was extracted using a simple maceration technique with ethanol and inhibition of 5α-reductase activities was determined. Solutions of 2% CT extract were loaded into four microemulsion (ME) formulas (F1, F2, F3 and F4) and investigated for their physical properties, skin permeation and stability. Results showed that crude CT extract had no toxic effects on DU145 cells at concentrations of 0.0001-1.0 mg/mL. For reduction of 5α-reductase activities, concentration of CT extract at 0.05 mg/mL exhibited highest 5-reductase type-1 inhibition activity on the DU-145 cell line at 89.96% of the control, higher than standard finasteride (31.39%) and dutasteride (38.58%). The results indicated that a thermodynamically stable microemulsion improved the stability and permeation rate of CT extract. Among the ME formula, F3 was most appropriate for ME formulation with highest permeation rate and good stability during 30 days of storage. Therefore, using nanotechnology for stable transdermal delivery systems of bioactive compounds from Thai medicinal plants is one approach to improve skin and hair follicle permeation.

Keywords: nanocarrier, skin permeation, microemulsion, *Carthamus tinctorius*

Formulation and characterization of clove oil microemulsions

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Abstract. The aim of this study was to develop clove oil microemulsions (ME) for topical application. ME containing clove oil as oil phase, Tween 80 as surfactants, ethanol as co-surfactant and water were formulated. The effects of surfactant:co-surfactant ratios (SR), the amount of clove oil and the amount of surfactant mixture (SM) on the physico-chemical properties (pH, conductivity, particle size, zeta potential, and thermodynamic stability) were evaluated. From pseudo-ternary phase diagrams constructed by using various SR (1:0.5, 1:1 and 1:2), the larger ME zone was found when the ratio was 1:2. The pH values of clove oil ME were 7-7.9. The droplet sizes of all formulations were 15-76 nm. Sizes and conductivities of the system were influenced by SR, the amount of clove oil and the amount of SM. The results revealed that clove oil formulation can be prepared using ME techniques. Topical o/w ME containing 5-20 % clove oil as oil phase with appropriated properties can be formulated using Tween80 and ethanol as surfactant systems.

Keywords: microemulsions, clove oil, Tween 80, ethanol, physico-chemical properties

Folate-functionalized amphiphilic chitosan polymeric micelles containing andrographolide analogue (3A.1) for colorectal cancer

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Abstract. A site-specific drug delivery system of anticancer agents has been delveloped to enhance the therapeutic efficacy and reduce toxicity to the normal tissue. Semi-synthetic andrographolide analogue 3A.1 (19-tert-butyldiphenylsilyl-8,17-epoxy andrographolide) is one of the potential natural anticancer compounds against many types of cancer including colorectal cancer cells. However, the clinical applications of this compound are limited because of low water solubility and lack of suitable delivery carriers. This study aimed to increase the aqueous solubility and improve the anticancer efficacy of 3A.1 via active targeting approaches. In this study, 3A.1 was loaded into the polymeric micelles self-assembled from N-naphthyl-N,O-succinyl chitosan (NSC). The micelles were conjugated with folate moiety (Fol-NSC) for targeting to the cancer cells. All of the 3A.1-loaded micelles were prepared by dropping method, and the physicochemical properties (size, charge, morphology, encapsulating efficiency, loading capacity), *in vitro* release behavior and *in vitro* anticancer activities against HT29 colorectal cancer cells were investigated. The 3A.1-loaded micelles were successfully formulated by dropping method using NSC or Fol-NSC. The micelles loaded with 40% initial 3A.1 showed the maximum encapsulating efficiency and loading capacity. The micelles were in the nanometer range, having a negative surface charge and a spherical structure. The colon site-specific release of the 3A.1 from the 3A.1-loaded micelles was obtained. The release of 3A.1 from the Fol-NSC micelles was slower than that from the NSC micelles. Moreover, the Fol-NSC micelles exhibited superior anticancer efficacy than that of the NSC micelles and free 3A.1. In conclusions, the 3A.1-loaded Fol-NSC micelles developed in the present study had suitable physicochemical properties. These nanocarriers may be a potential delivery system for targeted delivery of the 3A.1 to colorectal cancer cells.

Keywords: andrographolide, chitosan, polymeric micelles, folate, colorectal cancer

Evaluation of thermally crosslinked poly (acrylic acid-co-maleic acid) (PAMA)/poly (vinyl alcohol) (PVA) microneedle arrays

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Abstract. This study aimed to evaluate the optimal conditions for crosslinked PAMA/PVA microneedle (MN) arrays. Poly (acrylic acid-co-maleic acid) (PAMA)/poly (vinyl alcohol) (PVA) microneedle (MN) arrays were fabricated for the first time using the micromolding technique. PAMA/PVA MN arrays at the polymer ratio of 1:4 were sharp, homogenous and perfectly formed with an elegant appearance. The successful crosslinking MN arrays were determined using FTIR and water insolubilization. The results showed that increasing when crosslinking temperature and time were increased the degree of crosslinking also improved, which resulted in a decline in water uptake. The optimal crosslinking condition for PAMA/PVA MN arrays was 130°C for 1 h. Moreover, the highest swelling was observed from crosslinked PAMA/PVA MN arrays at 90°C for 0.5 h. These studies suggest that the combination of PAMA and PVA for fabrication of MN arrays could have much potential in the development of both hydrogel and dissolving MN devices for transdermal drug delivery.

Keywords: poly (acrylic acid-co-maleic acid) (PAMA), poly (vinyl alcohol) (PVA), dissolving microneedles, thermal crosslinking

Evaluation of anti-oxidant, tyrosinase inhibitory and anti-inflammatory activities of *Goniothalamus tavoyensis* chatterjee

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Abstract. *Goniothalamus tavoyensis* Chatterjee is one of the Goniothalamus genera in the Annonaceae, and has been distributed in Thailand. Previously, abortion, antiaging, body pains, rheumatism, skin complaints, typhoid fever, tympanites, stomach ache and fever are the main ideas of medicine supporting by these plant. Thus, the used of Goniothalamus genera in traditional medicine is well known. This study is to investigate for the potential on the antioxidant, anti-tyrosinase and anti-inflammatory activities of ethanol extract from leaves of *G. tavoyensis* (GNL-EtOH). The results showed the scavenge 1,1-Diphenyl-2- picrylhydrazyl (DPPH) and mushroom tyrosinase inhibitory activities with the IC_{50} values of 0.90 ± 0.01 mg/mL and 4.08 ± 0.13 mg/mL, respectively. Moreover, the GNL-EtOH reduced nitric oxide (NO) production in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophage cells with the IC_{50} values of 0.02 ± 0.01 mg/mL. These observations suggest that *G. tavoyensis* extract can further be utilized in cosmetic industries, food and traditional medicine applications.

Keywords: *Goniothalamus,* anti-oxidant, tyrosinase inhibition, anti-inflammatory

Electrostatic effects of metronidazole loaded in chitosan-pectin polyelectrolyte complexes

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Abstract. Electrostatic interactions of polymeric charges become one of the important factors to form the polyelectrolyte complexes (PECs). In this work, PECs has successfully created through the interaction between positive charges of chitosan (CS) and negative charges of pectin (PE) based on the effect of pH and pKa of the polymers. The formation of PECs provides small particle size, positive surface charge and high %entrapment efficiency (%EE) after loaded metronidazole (MTZ). Dropwise addition of PE solution into CS solution was carried out to form PECs. A certain concentration of chitosan and pectin fixed at ratio 3:1 while the pH of both polymers varied as pH 1, 3, 5 and 9. The alterations after forming PECs observed particle size, zeta potential and turbidity of the solution along with FTIR, DSC and TAG. Precipitation of PECs solution was found in the fixed pH 5 of PE solution dropwise into pH 7 and 9 CS solution, which referred to the unstable of the PECs system. The pH 1 and 9 of PE and CS obtained the large size which about 600-1200 nm, while zeta potential found a low positive charge of 5.54- 11.90 mV. Thus, only the fixed pH 5 of CS solution and pH 3, 5 or 7 of PE solution to form PECs were used to load MTZ. After loaded MTZ, the particle size of the PECs was about 400-500 nm and the zeta potential was about 20-50 mV. Electrostatic interactions resulted from FTIR detected the changes in amino groups of CS and carboxyl groups of PE. Thermal analysis on DSC for determinations of melting points or transition temperatures and TGA to monitor weight loss by heat were confirmed the PECs and MTZ-PECs formation. The pH 5 of PE interacts with pH 5 of CS offered the smallest particle size as 438 nm, the zeta potential about 23.5 mV and the highest percentage of EE as about 50% of the drug-loaded. The pH 5 of PE and CS were the pH-responsive to the pKa, thus, the acidity of the polymers may provide a suitable condition to form the appropriate polyelectrolyte complexes.

Keywords: electrostatic interaction, polyanion, polycation, polymeric charge, responsive pH and pKa

Effect of mucoadhesive substances on physico-chemical properties of in situ gels for buccal applications

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Abstract. In situ gels for oral mucoadhesive applications using benzalkonium chloride (BZK) and povidone iodine (PI) as active pharmaceutical ingredients were formulated using Poloxamer 407, a thermo-sensitive sol-gel polymer. Xanthan gum (XAN) and sodium carboxymethylcellulose (SCMC) were added to study the effect of mucoadhesive polymer on the physico-chemical properties of gels. The formulations were evaluated for the viscosity (25 and 37 °C), pH, gel strength in artificial saliva at 37 °C and adhesion to porcine buccal mucosa. At 37 °C, the viscosity of all preparations increased ($p<0.05$). They turned to gels in buccal conditions with good gel strength and dissolution time were more than 45 min. PI reduced the viscosity of the gel ($p<0.05$). For Poloxamer 407-PI formulation, only SCMC promoted the mucoadhesive property. In situ gels prepared from Poloxamer 407-BZK showed suitable properties for buccal applications. XAN and SCMC did promote the gel properties by increase the viscosity at 37 °C.

Keywords: In situ gels, buccal mucoadhesive, benzalkonium chloride, povidone iodine.

Effect of formulations and spray drying process conditions on physical properties of resveratrol spray-dried emulsions

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Abstract. Spray-dried emulsion is one of the useful strategies to enhance dissolution properties of poorly water-soluble drug for example resveratrol. Physical properties i.e. particle size and moisture content of spray-dried emulsions could affect their quality attributes. In this study, Box-Behnken design was performed in order to determine effect of formulation and spray drying condition parameters i.e. feed rate on responses including particle size and moisture content of resveratrol spray-dried emulsions. The spray-dried emulsions were prepared by varying content of low-methoxyl pectin (LMP) and caprylic/capric glycerides (CCG) and sprayed at different feed rate. Box-Behnken design results reveled that the particle size of spray-dried emulsions was significantly influenced by the content of LMP, interactions between LMP and CCG, interactions between LMP and feed rate. LMP content showed positive relationships with the particle size. The content of CCG had negative significantly effect on moisture content of the spray-dried emulsion. Mathematical models describing the relationships between studied parameters and responses provided good predictability. Based on model, the optimal formulation was prepared using 2.6% w/w of LMP, 9% w/w of CCG, and feed rate of 6.8 mL/min and the small particle size (~5.9 μm) and low moisture content (~5.6%) were obtained. The spray-dried emulsions were successfully prepared with satisfy quality. The Box-Behnken design would be an effective tool to elucidate influence of formulation and spray drying conditions on particle size and moisture content of the spray-dried emulsions. Further, the design aided in developing and optimizing the spray-dried emulsions with specified quality.

Keywords: resveratrol, spray-dried emulsion, Box-Behnken design, optimization

Dual-charge nanofiber mats made of chitosan (CS)/poly (vinyl alcohol) (PVA) and poly-(acrylic acid-co-maleic acid) (PAMA)/PVA

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Abstract. Nanofibers have been widely used for tissue engineering. Using charged polymers for the preparation of nanofibers can be useful for the loading of substances or macromolecules. Dual charge nanofiber mats are expected to be able to immobilize both positively charged and negatively charged substances in one versatile nanofiber mat. The purpose of this study was to prepare and characterize dual-charge nanofibers generated from poly (vinyl alcohol) (PVA)/poly-(acrylic acid-co-maleic acid) (PAMA) and chitosan (CS)/PVA. The polymer solutions of PAMA/PVA (1:1.63 w/w) and CS/PVA (1:2.33 w/w) were electrospun to form the nanofibers using dual-jet electrospinning process. The obtained dual-charge nanofibers were thermally crosslinked by leaving the nanofibers in the oven at 110-130 °C for 0.5, 1, 3, 5 h. The appearance of the nanofiber mat was characterized by a scanning electron microscope (SEM), and the diameter of nanofibers were determined by an image analysis software (J-micro vision®). The percentage water insolubilization and FT-IR spectra were also determined. The dual-size nanofiber mats with smooth and bead-free fibers were obtained. The diameter of the PAMA/PVA and CS/PVA fibers was 574.54 ± 142.98 nm and 225.69 ± 41.92 nm, respectively. The desirable temperature and time for the crosslink of the dual-charge nanofiber mats was 130 °C for 1 h which could provide a high insolubilization with water capacity of 93.22 ± 2.23%.

Keywords: nanofibers, electrospinning, dual charge, PVA, PAMA, chitosan

Development of topical vitamin E nanoemulsion

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Abstract. The objective of this research was to study the appropriate conditions for topical vitamin E nanoemulsion formulations such as types of instrument (Homogenizer and Ultrasonicator), power, mixing time, oil phase volume ratio, type and percentage of emulsifier in preparations. Nanoemulsion of higher percentage of oil phase, lower energy consumption and good physical appearance were selected for physical properties test. From the results of homogenizer method, particle size was reduced as increasing mixing time meanwhile ultrasonicator method exhibited more rapidly reduced particle size and produced more constant nanosize than homogenizer. The formulations with lower percentage of oil phase exhibited smaller size. The appropriate method was 75% amplitude of ultrasonicator with 40% v/v of oil phase mixed for 10 minutes. Types and percentages of emulsifier were varied as follow: span 80: tween 80 (ratio 7:3) at 5, 10, 15, 20% v/v; transcutol CG: tween 80 (ratio 7:3) at 5, 10, 15% v/v and sodium cholate at 0.5, 1, 2, 3, 5% w/v. Only formulations with span 80: tween 80 showed good physical properties without oil drop on the surface of emulsion. Stability studies were performed and interpreted by paired t-test with PSPP version 0.10.4. Nanoemulsion containing 5, 10 and 15% v/v of span 80: tween 80 were stable after 1 week storage at room temperature. The droplet size, zeta potential, pH and viscosity did not change significantly ($p \geq 0.05$).

Keywords: vitamin E, nanoemulsion

Development of Thai gac fruit extraction as a multifunctional cosmeceutical ingredient for antioxidant, melanogenesis and collagen stimulating activities.

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Abstract. Gac fruit (*Momordica cochinchinensis Spreng*) is mostly found in Southeast Asian countries. Previous studies have demonstrated that gac fruit is a major source of β-carotene, lycopene, and α-tocopherol. This study aimed to develop the extraction of Thai gac fruit as a potential active ingredient for antioxidant, melanogenesis, and collagen stimulating activities. In the present study, different parts of ripe Thai gac fruit, the peel, pulp, and seed membrane, were extracted using the accelerated solvent extraction (ASE) technique, an innovative plant extraction method. DPPH radical-scavenging activity, ABTS radical scavenging activity, and ferric reducing/antioxidant power (FRAP) assay were performed to compare the antioxidant activity derived from each part of the fruit. The effect of Thai gac fruit on melanogenesis and collagen stimulating activities was investigated using B16F1 and human fibroblast cell lines, respectively. The results demonstrate that the pulp (EC_{50} value of 7.42 ± 0.81 mg/mL) and the seed membrane (EC_{50} value of 76.43 ± 2.66 µg/mL) showed the strongest antioxidant activities in DPPH and ABTS assay, respectively. The peel clearly displayed the strongest antioxidant activity and significantly increased the FRAP value (247.67 ± 19.73 µM/mg). Furthermore, the peel showed the lowest melanin content (76.56 ± 7.13 % of control) in the B16F1 cell line, and clearly increased the most amount of collagen (21.87 ± 3.12%) in the human fibroblast cell line in comparison to the other fractions. Therefore, Thai gac fruit peel revealed the biological effects, including antioxidant, melanogenesis, and collagen stimulating activities. In conclusion, Thai gac fruit extracted using the ASE technique exhibited several effects that could be used as a potential active ingredient for cosmeceutical products, due to its antioxidant, melanogenesis, and collagen stimulating activities. Our findings provide potential uses of Thai gac fruit as a multifunctional cosmeceutical ingredient to be further explored in future studies.

Keywords: *Momordica cochinchinensis* Spreng, antioxidant, melanogenesis, collagen activity

Development of nanoemulsions containing coconut oil with mixed emulsifiers: effect of mixing speed on physical properties

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Abstract. Recently, coconut oil has become an attractive natural material consisting of monolaurin which exhibits antibacterial, antifungal, antiviral and antiprotozoal effects. However, coconut oil is relatively immiscible with water, the main composition of human body. This study was thus focused on the development of nanoemulsions containing coconut oil employing the combination of several surfactants and variation of mixing speed for the purpose of overcoming the immiscible problem. Formulations of nanoemulsions were prepared within this study. The HLB values were then measured and used to determine the compatibility between coconut oil and surfactants. Moreover, the speed of homogenizer which might have a significant impact on the physical properties of nanoemulsions, was taken into account. Meanwhile, particle size, zeta potential, pH, long-term stability and antibacterial activity were also examined. According to the results, the nanoemulsions prepared from 10% (w/w) of coconut oil and 10% (w/w) of surfactants comprising of Tween® 80 and Span® 80 at the ratio of 2 to 3 at the homogenization speed of 15000 rpm seemed to be suitable for topical administration with the characteristics as follows: the droplet size of 254.7±0.016 nm, the zeta potential of -4.41±1.46 mV and the pH values of 6.13± 0.01. In addition, coconut oil formulated in the form of nanoemulsions demonstrated the efficient antimicrobial activities against *Staphylococcus aureus*, a gram-positive bacteria. In conclusion, this study represents the effect of mixing speed on the properties of nanoemulsions containing coconut oil which could be further developed as a substitute for topical antibiotics.

Keywords: coconut oil, HLB, mixing speed, emulsifier, nanoemulsions, antimicrobial

Development of indomethacin and lidocaine sonophoresis gel

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Abstract. A transparent ultrasound gel of Indomethacin (IDM) combined with Lidocaine (L) was formulated to avoid gastrointestinal (GI) side effect from oral administration. Heat, co-solvent, eutectic mixture and solid dispersion (SD) methods were tested in order to circumvent IDM's aqueous insolubility in the transparent gel. The best method for this system was the solid dispersion method (IDM: polyethene glycol 4000, 1:20) together with co-solvent containing of isopropyl alcohol (IPA) and water. 15% Hydroxypropylmethylcellulose 5-9 cps (HPMC) was selected as a gelling agent. There was a chemical interaction between IDM and L from FTIR spectrogram. IDM was shown to be in an amorphous form in SD formulation from X ray diffractogram. After stability test, gel separation was noted when the gel was subjected to 4 cycles of temperature cycling at 4°C for 24 hours and 45°C for 24 hours. No significant change in physical appearance was found after the gel was kept at room temperature (30°C) for 4 days. However, the IR spectrum showed that the amount of IDM was significantly increased. This may be due to the interaction between some components in the formulation and/or vapor evaporation of the gel.

Keywords: indomethacin, lidocaine, sonophoresis, gel, formulation

Development of emulsions with anti-sebum secretion activity

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Abstract. The sebaceous glands exhibit the function of skin lubricant by releasing waxy oil, called sebum, into the hair follicles and to the surface of the skin. However, an excess secretion in sebum seems to be a major factor contributing to oily skin and acne formation. Most of the commercial products with anti-sebum secretion effect are mainly composed of synthetic chemicals which have the potential to cause allergic reactions. The objective of this study was to develop emulsions with anti-sebum secretion activity from natural extract substances, and were then investigated for the physicochemical stability and the inhibition effect on facial sebum production. Various formulations of oil in water (o/w) emulsions containing of green tea extract and L-carnitine were prepared. The obtained emulsions were examined for their stability including physical appearance, pH, viscosity, spreadability after storage under room temperature and accelerated condition. The selected preparations were then evaluated for their reduction effect on facial sebum secretion in participants for a period of 28 days. The results revealed that emulsions loaded with 2%w/w and 4%w/w of L-carnitine, as well as all emulsion bases, displayed good physical characteristics described as follows: a pH value of 5.80 to 6.88, a viscosity in the range of 1,377.67 cP to 2,143.67 cP, good spreadability and suitable stability under room temperature and accelerated condition. Nevertheless, the ability to penetrate the skin tended to be relatively low. Regarding to the study of the reduction effect on facial sebum secretion in volunteers, 16 and 17 from all of 17 volunteers are effective on facial sebum secretion from emulsions loaded with 2%w/w and 4%w/w of L-carnitine, respectively. Therefore, the information obtained from the development of emulsions is expected to be used as a guideline for further improvement of the formulation.

Keywords: formulation, emulsion, oily skin, anti-sebum secretion activity

Development and evaluation of hydroxypropyl methylcellulose patches containing clindamycin for topical application

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Abstract. Clindamycin (CM) is the one of antibacterial drugs that can be used to treat acne vulgaris. The commercial products in form of solutions, creams, and gels cannot provide the exact amount of the drug and constant drug release. Transdermal patches present an attractive point for reducing this limitation and there is no commercial transdermal patch containing CM available in the market nowadays. The purposes of this study were to develop CM loaded transdermal patches for the treatment of acne and to investigate the physical properties and drug release profile of the CM from the transdermal patches. The transdermal patch was prepared using 10% HPMC. The types and concentrations of additives (glycerin, polyethylene glycol(PEG) or propylene glycol (PG)), were varied to improve the properties of the patches. The physical appearances including the translucent, color thickness and weight of the patches were recorded. The mechanical properties and skin adhesion of the patches were determined by a texture analyzer. The polymorphism of CM in the patches and the release profile of CM from the patches were investigated by X-ray diffraction and Franz diffusion cell, respectively. CM transdermal patches were translucent. The weight and thickness of the patches increased as the amount of additive increased. Glycerin and PG decreased the strength of the patches, while PEG increased the hardness. Adding CM to the patches increased the hardness and decreased the elasticity of the patches. The internal structure of CM loaded into the patches was an amorphous form. The CM patches exhibited some adhesion properties when contacted with the porcine skin. The release of CM from the patches was found to be 71-108% within 60 minutes. The patch prepared from 10% HPMC, 15% Glycerin, and 5% PG displayed the highest release rate. In conclusion, the CM loaded HPMC patches presented desirable properties, which could be used as a transdermal patch for the treatment of acne.

Keywords: clindamycin hydrochloride, transdermal patches, drug release

Development and characterizations of amphotericin B nanoemulsion containing cyclodextrin

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Abstract. Fungal keratitis, a corneal fungal infection of the eyes caused mainly by *Candida, Fusarium* and *Aspergillus* species, has become the leading cause of blindness from corneal disease. Amphotericin B (AmB) is considered as the drug of choice for fungal infections. However, its use in ophthalmic drug delivery is limited by low corneal residence at ocular surface as a result of blinking reflex, tear turnover and nasopharyngeal drainage. The aim of this study was to demonstrate AmB/cyclodextrin (CD) complex loaded nanoemulsion for the improvement of targeted delivery of AmB to the ocular surface. AmB/CD loaded nanoemulsion was prepared by using MCT as lipid phase and lecithin as emulsifier with high pressure homogenizer. The pH, osmolality, viscosity, particle size, zeta potential, drug content, entrapment efficiency, the aggregation behavior of AmB and the stability index were evaluated. The prepared nanoemulsions exhibited a measured size range of 181-425 nm, zeta potential of 30-32mV and entrapment efficiency of 75-80%. All formulations exhibited AmB in moderate aggregated form. Thus, it supported that AmB nanoemulsion could be a promising system for effective ocular drug delivery of AmB for targeted fungal keratitis therapy.

Keywords: amphotericin B, cyclodextrin, nanoemulsion, fungal keratitis

Design of caffeine-loaded nanostructured lipid carriers containing coconut oil for topical application

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Abstract. Although caffeine was suggested as one of the pharmacological agents for the cellulite treatment, its skin permeation restricted. The present work was aimed at formulating caffeine loaded nanostructured lipid carriers (CAF-NLCs) containing coconut oil as a topical delivery system. CAF-NLCs were prepared by the ultrasonic emulsification method, using coconut oil as a liquid lipid. The proper selection of solid lipid and surfactants for these formulations were investigated. Subsequently, physicochemical properties, entrapment efficacy, stability, and *in vitro* drug release were evaluated. The CAF-NLCs containing coconut oil was successfully prepared using glyceryl behenate as a solid lipid and showed an interesting entrapment efficiency (62-99%). The obtained CAF-NLCs presented the nanosized range (≈ 60-390 nm), with a low polydispersity index and high negative zeta potential values (over -30 mV). However, type and concentration of surfactant also affected these properties. These results suggested that CAF-NLCs containing coconut oil are the promising carrier for delivery of caffeine following topical application.

Keywords: caffeine, nanostructured lipid carriers, coconut oil, encapsulation

Comparison between batch and fed-batch fermentations to produce human monoclonal single-chain antibody variable fragments target to influenza virus NS1 protein

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Abstract. Influenza is a highly contagious respiratory disease that can lead to severe, life-threatening clinical manifestations. The causative agent of the disease is influenza virus which is classified into four types, A, B, C, and D. However, only the type A influenza virus has epidemic and pandemic potential. Although influenza is a vaccine preventable disease, and there are direct-acting drugs for treatment, several limitations are concerned such as the production and use of the vaccines, and the effectiveness of the chemical drugs. Recently, human monoclonal single-chain antibody variable fragments (HuscFv) that bound to recombinant and native non-structural protein-1 (NS1) of influenza A virus were successfully produced by using a phage display technology. The HuscFvs produced from NS1-bound-phage-transformed *Escherichia coli* clones in laboratory scale showed replication inhibition of the type A influenza virus. Therefore, the aim of this study was to produce the anti-influenza HuscFv in large scale by comparing between batch and fed-batch fermentations. The results showed that fed-batch fermentation of *E. coli* producing HuscFv in a 10-L-bioreactor gave higher cell density and dry cell weight (DCW) at all time intervals after IPTG induction compared to batch cultivation. Interestingly, fed-batch fermentation gave 109.4 g/L (wet weight) of bacterial cells, while only 72.5 g/L (wet weight) of bacteria were harvested by batch fermentation. Although the plasmid stabilities of bacterial producer were still high in both methods after IPTG induction, the level of HuscFv expression was very low for batch cultivation. However, the level of HuscFv expression slightly increased at 1, 2, 3, and 4 hours after IPTG induction when the bacterial producer was cultivated by fed-batch fermentation. The bacterial producer expressed the influenza NS1-specific HuscFv as an insoluble form seen as band at approximately 26 kDa after SDS-PAGE and CBB staining. Data presented here highlights that the fed-batch cultivation was superior to the conventional batch cultivation in productivity of the NS1-specific HuscFv.

Keywords: batch fermentation, fed-batch fermentation, human monoclonal single-chain antibody variable fragments (HuscFv), influenza A virus, NS1 protein

Comparative study of oryzanol- and rice bran oil-load niosomes for anti-aging cosmetics

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Abstract. Oryzanol (OZ) is one of such a phytochemical purified from rice bran oil (RBO). Tocopherols, tocotrinols, sterols, fatty acids, vitamin A, vitamin B and lecithin are also ingredients having the potential to be used as emollient and antioxidant in pharmaceutical, nutraceutical and cosmoceutical products. Recently, the nanotechnology focused on the production of OZ loaded niosomes. The simultaneous incorporation of various components of RBO into niosomes was compared to pure OZ formulation. The aim of this study was to develop the oryzanol- and rice bran oil-load niosomes for anti-aging cosmetics. The of niosome formulation from rice (OZ and RBO) was conducted by sonication method. The physicochemical properties and stability of the formulation were the parameters that indicated the production ability of niosome formulation. The vesicle size, polydispersity index, zeta potential and entrapment efficiency were investigated. The results indicated that OZ- and RBO-loaded niosome were in the nanosize range 83 to 203 nm. The polydispersity index was 0.22 to 0.44. The zeta potential was negative between 8 to 32 mV. The entrapment efficiency was 4.2 to 52.7%. The OZ- and RBO-loaded niosome were stable under 5 and 25 °C for 90 days. The finding suggests that physicochemical properties of the RBO-loaded niosomes were close to the OZ-loaded niosomes. The skin permeation and antioxidant activity are necessary to be conducted in further study.

Keywords: rice bran oil, oryzanol, anti-aging, nanovesicles, niosomes

Characteristic assessment of the polymeric films used for hair gel products in Thailand

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Abstract. The market of hair gel products in Thailand steadily grows with the changing trend of fashion every year. The main component of the hair gel products is the fixative polymer, which plays a role in hair styling properties including stiffness and curl retention. Properties of the hair styling product depending on hair types and climates must be suitably improved for Thai hair. In this study, 10 %w/w gel solutions of polyacrylic acid, polyquaternium-86, polyvinylpyrrolidone (PVP), acrylates copolymer, and vinylpyrrolidone (VP) copolymers were cast as polymeric thin films. The film properties such as mechanical properties, wettability, and crystallinity were characterized afterwards. As a result, the highest puncture strength of 2.2 N/mm^2 was found from PVP-K90 film (500-700 μm thick). Whereas, VP/vinyl acetate (VA) copolymer film was the most flexible with 42.5% elongation. For water contact angle analysis, it was noticed that VP copolymers had the angle of ~50^o indicating the moderate wettability. The x-ray patterns of raw materials, polymeric films, and humidified films were varied especially for PVP-K30. Morphologies of the Thai hair samples coated with the fixative polymers were visualized through SEM. After storing coated hair samples in the humidified atmosphere (95±2% Relative humidity), acrylates copolymer, VP/Methacrylamide/Vinyl Imidazole copolymer, and VP/VA copolymer appeared the humidity resistance suitable for Thai hair styling gel.

Keywords: fixative polymer, polymeric film, mechanical property, water contact angle, humidity

Catechol-functionalized succinyl chitosan for novel mucoadhesive drug delivery

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Abstract. The objective of this research was to synthesize and assess a catechol-bearing succinyl chitosan (Cat-SCS) as an innovative mucoadhesive substance for a mucoadhesive drug delivery system. Succinyl chitosan (SCS) was synthesized *via* ring-opening reactions with succinic anhydride. The Cat-SCS was then synthesized by reacting SCS with dopamine with the existence of N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDAC) and N-hydroxysulfosuccinimide (NHS). The successful functionalization of catechol onto the chitosan backbone was verified using nuclear magnetic resonance spectroscopy (NMR) and Fourier transform infrared spectroscopy (FT-IR). Mucoadhesion studies were performed using rheology measurement and *ex-vivo* mucoadhesion test. The findings exposed that the synthesized Cat-SCS exhibited excellent mucoadhesive properties which was better than the intact CS. Further indirect studies verified the occurrence of polymer-mucin glycoproteins interactions. The catechol content on the Cat-SCS was determined to be 0.377 using ^{1}H NMR. The cytotoxicity test indicated the non-noxiousness of the obtained polymer on a fibroblast cells (HGF cells). Therefore, these results could advocate the capacity to use Cat-SCS as an inventive mucoadhesive platform for mucoadhesive drug delivery.

Keywords: catechol, chitosan, dopamine, mucoadhesive polymer

Biological activities and dermal penetration of liposome-containing *Coprinus atramentraris* extract

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Abstract. The total phenolic content and antioxidant activities of five edible mushrooms (*Pleurotus djamor, Agaricus bisporus, Hericlum erinaceus, Coprinus atramentraris and P. ostreatus*) were evaluated. The *Coprinus atramentraris* ethanolic extract (CE) showed the promising antioxidant property and had a strong relationship with phenolic compound content. Liposome-containing CE formulations, were prepared with various lipid composition. The liposome formulation, L1, which composed of phospholipids and cholesterol at ratio of 6:4 gave the highest entrapment efficiency (24.03%), small particle size (143.03 nm) and favorable particle size distribution (0.311) with small values of zeta potential (-30.2 mV). L1 showed good anti-elastase property (IC50 0.029) and no cytotoxicity effect to the human skin fibroblasts and melanoma cells even at the highest concentration of 1 mg/mL compared to the unformulated extract. The in vitro skin permeation studies, using human cadaver skin and modified Franz-diffusion cells, showed that the L1 was able to considerably increase the rate of permeation of phenolic compounds in L1 compared to the CE solution. These results reflect the use of L1 as active agents in cosmeceuticals.

Keywords: mushroom, *Coprinus atramentaris*, liposome, skin permeation, biological activities

Application of simplex lattice design in formulation development of lozenges containing *Vernonia cinerea* extract for smoking cessation

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Abstract. The aim of this work was to apply the simplex lattice design in the formulation development of lozenges containing *Vernonia cinerea* extract. The different ratios of three diluents; milk powder, xylitol, and inulin were investigated. The preformulation parameters of powder mixtures were evaluated. The compressed lozenges were evaluated for their weight variation, thickness, hardness, friability, and disintegration time. Results showed that the ratios of the three diluents affected the preformulation parameters. The angle of repose data revealed that all 12 powder mixtures had excellent flow property. The formulation containing milk powder had the highest values of Carr's index and Hausner ratio, indicating the poor compressibility. While formulation containing inulin had the best compressibility. A formulation containing xylitol had the lowest moisture content. The compressed lozenges had a weight variation of less than 5%, a diameter of 1.5 mm, a thickness of 5-6 mm, and a hardness of 2-12 kP. The 8 of 12 formulations had friability of less than 1%. Use of milk powder provided the longest disintegration time. The desired properties of developed lozenge achieved when the medium amount of xylitol and inulin and low amount of milk powder were used. The optimal diluent ratio providing the weight variation not more than 5%, the hardness of 5-8 kP, friability not more than 1%, and disintegration time not more than 30 min was an equal weight ratio of milk powder, xylitol, and inulin.

Keywords: simplex lattice design, lozenge, *vernonia cinerea*, diluent, smoking cessation

Anti-melanogenic activity of *p*-chlorophenyl benzyl ether in α-MSH-induced mouse melanoma B16F10 cells

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Abstract. Melanin is cutaneous pigment which level of its production determines skin complexion. Overproduction of melanin, frequently promoted by UV rays, results in darkening of the skin. Inhibition of tyrosinase activity, a core component in melanin biosynthesis, is one of the mechanisms of depigmenting agents. Hydroquinone and kojic acid are the examples of well-known whitening agents widely used in both pharmaceutical and cosmetic products. However, their adverse effect issues still needed to be overcome. A recent study showed that *p*-chlorophenyl benzyl ether (Cl-benz), a new synthetic compound, more strongly inhibited mushroom tyrosinase than kojic acid. In the current study, cytotoxicity, anti-melanogenic activity and tyrosinase inhibitory activity of Cl-benz were performed in mouse B16F10 melanoma cells compared to kojic acid. After 24 h of treatment on B16F10 cells, the cytotoxicity was not observed with Cl-benz and kojic acid. However, after incubation for 48 h, kojic acid at a concentration of 500 μM reduced cell viability lower than 50%, whereas Cl-benz-treated cells showed negligible cytotoxicity. For cell-based assay, Cl-benz exhibited anti-melanogenic activity and tyrosinase inhibitory activity similar to kojic acid. Melanin production in B16F10 cells was suppressed by Cl-benz in a dose dependent manner. One hundred micrograms of Cl-benz decreased melanin content in α-MSH-stimulated cells by 66%. Moreover, cellular tyrosinase activity showed a positive correlation with melanin content in Cl-benz-treated cells. These results revealed that Cl-benz could inhibit melanogenesis via the mechanism of cellular tyrosinase inhibition. Accordingly, Cl-benz has potential to become a novel skin whitening agent in terms of efficacy and safety.

Keywords: melanin, tyrosinase, anti-melanogenic activity, whitening agent, *p*-chlorophenyl benzyl ether

Andrographolide-loaded nanoemulsion and its activity against non-melanoma skin cancer cells

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Abstract. Andrographolide (AG) is a diterpenoid lactone found in *Andrographis paniculata* leaves and stems. It has excellent activity against various cancer cells, for example, skin cancer cells. However, application of AG for skin cancer treatment in clinical trials is limited due to its poor water solubility. To overcome this problem, oil in water AG-loaded nanoemulsion (AG-NE) would be prepared. The objectives of this study were to investigate physicochemical properties of AG-NE and to determine its activity against non-melanoma skin cancer cells. Nanoemulsion (NE) without AG (NE base) and NE containing AG (0.1%w/w) were prepared by high-pressure homogenization technique. They contained a mixture of Tween 80 and Span 80 (5:1) (10% w/w) as an emulsifier. Their droplet size, zeta potential and physical stability were evaluated. Cytotoxicity of AG and AG-NE to non-melanoma skin cancer cells (A-431 cells) and normal skin fibroblast cells (HFF-1 cells) were investigated. The results showed that NE base and AG-NE had droplet size in a nanometer range. They had low viscosity with the flow behavior consistent with Newtonian liquids. Although their zeta potential values were slightly low, they showed good physical stability against centrifugal force. AG and AG entrapped in AG-NE were not toxic to HFF-1 cells at low concentrations. They could induce apoptosis of A-431 cells with IC_{50} of 25.83 μg/ml and 54.80 μg/ml, respectively. Therefore, AG-NE has become possible to use for investigation of its efficacy and safety in animal models and clinical trials.

Keywords: andrographolide, nanoemulsion, non-melanoma skin cancer, apoptosis

A comparison of antioxidant capacity and total polyphenols quantity between imported olive oils and edible vegetable oils in Thailand

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Abstract. This study aimed to compare the total polyphenols and antioxidant capacity between imported extra virgin olive oil (Evoo) and seven types of edible vegetable oils in Thailand. Seven types of edible vegetable oils produced and marketed in Thailand, was coconut oil (Co), palm oil (Po), olive oil (Oo), soybean oil (Sbo), sesame oil (Seo), rice bran oil (Rio), and sunflower oil (Suo), were evaluated the level of total polyphenols content and antioxidant capacity. Two brands of each seven types of edible vegetable oils marketed in Thailand were compared to three brands of imported extra virgin olive oil (Evoo) for every test. Folin-Ciocalteau method proceeded to quantify the total polyphenols in eight edible vegetable oils. DPPH radical scavenging assay used to provide IC_{50} values of the samples. Considering the results from this study, we found that the methanolic extracts of brand E of Evoo allowed the highest average amount of total polyphenols of 9.26 mg GAE/g crude extract and IC_{50} values of 24 ppm. These comparisons indicate that the difference of gallic acid equivalent content and IC_{50} values connected to the types of edible vegetable oils whereas no correlation between gallic acid equivalent content and IC_{50} values, with statistically significant ($p\text{-}value < 0.05$).

Keywords: olive oils, edible vegetable oils, DPPH, total polyphenols, antioxidant activity

Solid lipid nanoparticles containing *Pueraria mirifica* ethanolic extract for hair growth promotion

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Abstract. *Pueraria mirifica* (PM) extract is locally used to promote hair growth. However, the effective transdermal delivery system should be prepared to deliver the extract through the skin barrier. The objective of this study was to develop solid lipid nanoparticles (SLN) containing PM ethanolic extract for hair growth promotion. The cell viability and proliferation of human follicle dermal papilla cells (HFDPCs) treated with PM extract were evaluated by MTT assay. SLN formulations were developed as a transdermal delivery system of the PM extract, compared with liposomes. The physicochemical properties of these nanoparticles were determined. The *in vitro* skin permeation study was also evaluated by Franz type diffusion cells. For the result, PM extract was a good safety herbal extract, which no cytotoxicity at the concentrations from 1 to 1,000 μg/ml. The cell proliferation of PM extract treated HFDPCs significantly increased in a dose-dependent manner, indicating the possibility to promote hair growth at the concentrations from 10 to 100 μg/ml. For formulation development, 5% (w/v) PM extract-loaded SLN exhibited small particle size (93.83 ± 0.32 nm) with narrow size distribution and negatively charged. This formulation had the highest percent entrapment efficiency (42.64 ± 0.47%), followed by SLN containing 1% (w/v) PM extract (8.84 ± 0.24%) and undetectable in liposomes. For the skin permeation result, SLN containing 5% (w/v) of PM extract could penetrate through the skin more than solution form. Therefore, the small particle size and high PM extract entrapped in SLN exhibited higher PM extract penetrated through the skin barrier and hair follicles than PM ethanolic extract solution. PM extract-loaded SLN might be an effective formulation for hair growth disorders treatment.

Keywords: *Pueraria mirifica*, dermal papilla cell, hair growth, solid lipid nanoparticle, transdermal

In vivo toxicity testing and clearance of gold nanoparticles in whole blood and urine samples of animal models

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Abstract. Gold nanoparticles (AuNPs) are a promising candidate for *in vivo* applications given their low immunogenicity, clearance times, and biocompatibility. AuNPs have been widely employed for diagnostics, and have seen increasing use in the area of therapeutics. Before applying AuNPs to the clinical level requires rigorous testing and inspection in order to reduce toxicity and unnecessary impact. Animal studies using ICP-MS analysis of blood and urine have shown that gold nanoparticles cause no morbidity at any concentration up to 1x10E13 particles/injection. Urine analysis showed a significant percentage of particles are cleared through renal filtration within 1 h, continues for up to 24 h and returns to baseline concentrations within 1 week. Whole blood analysis showed particle circulation within the bloodstream up to 24 hours post-injection. This was consistent with the primary clearance pathway of the particles being by excretion in urine. Gold nanoparticles also targeted primary organs although providing gradual dissipation and clearance over time. In summary of all study, mice injected with gold nanoparticles did not experience any clinical signs of illness, stress, or discomfort, nor did any expire over the course of the entire 6-week study. Therefore, gold nanoparticles presumably did not cause any toxic effects and undetectable stress to the mice. This study suggests that gold nanoparticles may be as promising materials to construct nanoscale diagnostic and therapeutic delivery systems.

Keywords: gold nanoparticles, animal models, ICP-MS analysis, diagnostic and therapeutic

Formulation of vapour patch containing green shallot oil

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Abstract. Vapour patch belongs to a novel dosage form inhalation formulation in the market. Essential oils are release from this patch up to 6 h and they are used only in cases of nasal decongestion mainly. In this type of patch, the adhesive layer serves not only to adhere the various layers together but also to release essentials oils flavor. In this present study, optimized concentration of four essential oils (green shallot, lemongrass oil, geranium oil, and ylang-ylang oil) was embedded into the ethylcellulose (EC) and triethylcitrate (TEC) polymers to develop vapour patch type nasal decongestion formulation. The developed formulations were characterized for physico-chemical properties, essential oils release efficiency and essential oil-polymer interaction. The vapour patches developed and evaluated currently were smooth, homogenous, and transparent and provided excellent vaporize activity after 12 h.

Keywords: green shallot, lemongrass oil, geranium oil, ylang-ylang oil, ethylcellulose, triethylcitrate

Formulation and evaluation of antifungal shampoo containing modified coconut oil for tinea capitis treatment

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Abstract. Modified coconut oil (MCO) has been reported as a promising material for eradication of various fungi which are the primary causes of tinea capitis. The antifungal shampoo is reported as an effective adjuvant therapy for fungi infection. In order to develop MCO as an active ingredient in antifungal shampoo, formulation factors affecting the physical and antifungal properties of shampoo including type and amount of surfactants were investigated. The results indicated that formula containing triethanolamine lauryl sulfate (TLE), ranging from 40-70% w/w, showed the most transparency and stable among the primary surfactants used, suggesting the good compatibility with MCO. An addition of polyoxyethylene 20 sorbitan monolaurate (PSL), an auxiliary surfactant, in the range from 5-40%w/w could enhance the clearness of shampoo while the suitable amount of PSL is also depended on TLE concentration. Various surfactant mixtures of TLE ranging from 40-60%w/w and 20-40% w/w of PSL were selected to formulate MCO shampoo. All formulations of shampoo showed high transparency, effective cleaning action, good stability and Newtonian flow behavior. Moreover, all formulations could inhibit *T. rubrum* and *M. canis* both before and after temperature cycling test. Therefore, this study revealed good feasibility of MCO shampoos for tinea capitis treatment.

Keywords: modified coconut oil, antifungal, shampoo, *Microsporum canis, Trichophyton rubrum*

Screening of UVB-protective effects of *Citrus maxima* and *Citrus hystrix* extracts on human keratinocyte cell line

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Abstract. UVB radiation is one of the major causes of a variety of skin disorders including sunburn, skin inflammation and skin cancer. At present, UVB protective effects of several citrus species have been reported. However, these reports are mostly studied in citrus plants indigenous to other countries, while UVB-protective activities of citrus species endemic to Thailand are largely unexplored. This study aimed to evaluate UVB protective effects citrus plants endemic to Thailand including pomelo (*C. maxima*) and kaffir lime (*C. hystrix*). Four different types of extracts i.e. pomelo juice, pomelo peel, kaffir lime juice, and kaffir lime peel extract, were tested. The results revealed that the extracts did not exhibit cytotoxic effect toward human immortalized keratinocyte cell line HaCaT even at high concentration (500µg/mL). However, a slight increase in cell viability was observed in most extract treated cells at 24h, which become less pronounced at 48h, suggesting that these extracts may have transient growth-stimulatory activities. For UVB-protective studies, HaCaT cells were pretreated with extracts for 4 hours, then replaced the media with PBS and exposed to UVB for 3 minutes, then maintained the cells in media containing extracts for 24 hours. Afterward, cell viability was analyzed through an MTT assay. The results indicated that pomelo juice extract demonstrated the highest potential in the reduction of UVB-mediated decrease in HaCaT cell viability. In conclusion, our screening results highlight pomelo juice as a candidate for further UVB-protective investigations.

Keywords: HaCaT, UVB, pomeo, kaffir lime, *Citrus maxima*, *Citrus hystrix*, UV protection

Physicochemical characteristics and antioxidant activity of mak mao berry loaded nanovesicles for cosmetics

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Abstract. Currently, nanotechnology is continuously utilized to improve the therapeutic effect of a natural antioxidant. The objective of this study was to design and develop the natural antioxidant loaded nanovesicle (liposomes and niosomes). Antidesma puncticulatum extract or maoberry (MM) was used as an active ingredient. MM obtained from Pra-Arjarn-Fhan-Arjaro Hospital, Sakon Nakhon. MM loaded nanovesicle was prepared by sonication method. The vesicle size, size distribution, zeta potential and total organic acids was investigated. Moreover, the antioxidant activity and total phenolic content were evaluated by radical scavenging assay (DPPH) and Folin Ciocalteu method, respectively. The vesicle size of MM loaded liposomes and niosomes was 94-550 and 116-687 nm, respectively. The width of size distribution was 0.27-0.81 and 0.46-0.93. While the zeta potential was 2-21 and 6-24 (-mV). The ascorbic acid was an antioxidant that determined by the HPLC analysis. The oxalic acid, malic acid, citric acid and tartaric acid were organic acid presented in MM loaded nanovesicles. The inhibition was liposomes and niosomes was 23-62 and 23-42%, respectively. The total phenolic compound was 70-132 and 88-182 mg gallic acid equivalent/mg (or mg GAE/g). The finding could be concluded that the source of natural antioxidant as MM can be incorporated in the nanovesicles as liposomes and niosomes. To improve the market value of the MM extraction from Pra-Arjarn-Fhan-Arjaro Hospital, further study is required to incorporate MM loaded nanovesicle into serum base.

Keywords: mak mao berry, berry extract, liposome, niosome, total phenolic

Gamboge resin-based phase separation *in situ* forming gel

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Abstract. The *in situ* forming gel (ISG) has been developed as a controlled drug delivery system to prolong drug release. This study aims to prepare ISG system based on gamboge resin (GB) as matrix former. The various amounts of GB dissolving in dimethyl sulfoxide (DMSO) and *N*-methyl pyrrolidone (NMP) were used to obtain the ISG systems and evaluated for their pH, viscosity, injectability and matrix forming. The antimicrobial activities against three strains of *Staphylococcus aureus* including the Methicilin Resistant *S. aureus* (MRSA) and *Porphyromonas gingivalis* were conducted. GB ISG systems exhibited good injectability with low viscosity. GB in DMSO showed a faster transformation to solid matrix compared to that in NMP. The gambogic acid (GA) content in crude GB was 70.33± .%13.0 The sustainable GA release from GB ISG could be attained. Thus, GB ISG exhibits the potential application as a drug delivery system for active compounds and is enable for using as a therapeutic dosage form owing to its antimicrobial activity.

Keywords: gamboge resin; phase separation; *in situ* forming gel

Preparation of spray containing essential oils of *Ocimum basilicum* and *Plectranthus amboinicus* (family lamiaceae) and determination of mosquito repellent activity against *Aedes aegypti*

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Abstract. Dengue is the most abundant mosquito borne disease. According to World Health Organization (2013), 50-100 million of people and 2.5 % affected children are suffering from severe dengue which later on become the cause of their death. Mosquito repellents are significantly known for personal protection against mosquito bites. There are many available synthetic repellents, which mostly composed of DEET (N, N-Diethyl-meta-toluamide), and Picardin which can cause health hazards that could lead to serious problems which necessitates effective yet safer alternatives. Basil and oregano are known to contain volatile oils that are potential insect-repellent agents and can be used as active ingredients of topical spray. The volatile oils were extracted using water distillation method and were screened for compatibility with excipients of topical spray formulation using differential scanning calorimetry were found to be compatible with sodium lauryl sulfate, propylene glycol, stearic acid, methyl and propyl parabens. Three topical spray formulations were prepared using the combined basil and oregano oil as active ingredients and were subjected to stability testing. The most stable formulation, which contains 1% combined essential oils basil and oregano, was further subjected to dermal irritation studies (Draize skin test) and mosquito repellency determination through cone test method. Based from the two-factor repeated measures ANOVA analysis, 0.25 mL and 0.5 mL of the formulated topical spray showed significant differences in their % knockdown rate across different time periods but there was no significant difference in their % knockdown activity. In addition, positive control (Baygon spray) showed significantly higher % knockdown rate compared to the formulated topical spray using Two-factor ANOVA. The formulated topical mosquito spray was found to be too strong as a mosquito repellent but rather an insecticidal spray that can be a safer and good alternative to commercial products since it is stable and cannot cause skin irritation.

Keywords: mosquito repellency, insecticidal, oregano, basil, topical spray

Development of coated tablets using polymer blend technique

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Abstract. This is a research to develop film coated tablets with acid resistant properties and ability of sustained release in the condition of small intestine by polymer blend technique. The research aimed to develop controlled-release coated tablets and to compare rheological, mechanical properties, disintegration and drug release of coating polymer between using single polymer and polymer blends. Theophylline and chlorpheniramine maleate (CPM) were incorporated into core tablets and used as model drugs. The core tablets were prepared by wet granulation. The core tablets were coated with hydroxypropyl methylcellulose (HPMC), Eudragit® L, ethyl cellulose (EC) and their blend. 10% Triethyl citrate (TEC) was used as a plasticizer. Thai coater was used for coating tablets. Rheometer was used for investigating rheological properties. Texture analyzer was used for investigating mechanical properties Dissolution test and Disintegration test were studied in 0.1 N HCl and phosphate buffer pH 6.8 medium. The result showed that the polymer blend solutions had lower viscosity than HPMC solution. However, the rheological properties have no difference between the polymer solution and polymer blend solutions. The Increasing temperature was to change viscoelasticity of Eudragit® L solution. The temperature of changing from viscous to elastic properties of polymer blend solutions between Eudragit® L and HPMC was increased. Film mechanical properties could be studied only with HPMC films. The other polymers and polymer blends were fragile and immiscible films. Polymer blends coated tablet have slower disintegration time in both medium. Theophylline release from the polymer blends coated tablets showed slower drug release than the single polymer coated tablets in both medium. The polymer blends between HPMC and EC in the ratio of 1:1 resulted in no drug release in 0.1 M HCl and slow release in phosphate buffer pH 6.8. In contrast to theophylline, CPM release was faster release and independent on pH. It can be concluded that the polymer blend in the appropriate ratio can achieve controlled-release tablets and provide rheological properties.

Keywords: coated tablets, polymer blend, controlled-release, rheological properties

Development and evaluation of acetaminophen orodispersible disc with taste-masking property

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Abstract. Orodispersible tablet (ODT) provides rapid disintegration in the oral cavity. ODT improves the efficacy of using medicines especially in term of compliance in a patient with dysphagia. The major challenge of ODT is a bitter taste of active pharmaceutical ingredient (API); they can lead the patients to refuse medication. This study focused on the developing of the orodispersible disc (ODD) with a taste masking property by preparation of API particle that was taste-masked by polymers. The co-precipitation technique was used for the taste masking. Acetaminophen (APAP) taste-masked particles prepared with different grades of crospovidone were investigated for the taste-masking property. Kollidon® CL-SF exhibited the lowest drug release. An increasing amount of Kollidon® CL-SF caused increasing of APAP release. Particles consisted of APAP:crospovidone at ratio 3:1 showed the lowest drug release. It was probably due to the complexation which was shown by the FT-IR and DSC. Taste-masked particles were compressed as ODD by a hydraulic press machine. The disintegrating time of ODD was at the range of 12.62-17.35 s. The dissolution of ODD was focused on the taste-masking zone (drug release in 1 min). The ODD with ratio 3:1 particle (APAP: Kollidon® CL-SF) showed the highest taste-masking performance, the lowest drug release. All disc formulations completely released APAP after 30 min. Therefore, the preparation of ODD contains taste-masked particles was promising to improve patient compliance of bitter APIs.

Keywords: acetaminophen, co-precipitation technique, crospovidone, orodispersible tablet, taste-masking property

Characterization of lauric acid precipitated from biocompatible solvents

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Abstract. Water tolerance of lauric acid (L) dissolved in biocompatible solvents mainly depended on the water affinity of solvent. L in DMSO (DL) was the most sensitive to water, higher than L in *N*-methyl pyrrolidone (NL) and L in 2-pyrolidone (PL), respectively. From scanning electron microscope, differential scanning calorimetry, thermogravimetric analysis, powder x-ray diffractometry and hot stage microscope tests revealed the alteration of L crystal owing to the interference by solvent during precipitation. All L precipitates had lower melting point and degradation temperature than intact L in which L precipitated from 2- pyrrolidone exhibited the lowest melting temperature. These characteristics will be useful for modifying L in phase inversion induced *in situ* forming matrix.

Keywords: lauric acid, precipitate, characterization, biocompatible solvent

Antioxidant, antityrosinase activity and toxicity of *Alpinia nigra* extracts

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Abstract. Excessive of ultraviolet light causes abnormality of melanin production. Antioxidants and antityrosinase agents are able to reduce hyperpigmentation by interrupting the process of melanin production. The purpose of this study is to examine the antioxidant and antityrosinase activities as well as toxicity of both 80% ethanol and aqueous extracts of *Alpinia nigra* by DPPH free radical scavenging assay, mushroom tyrosinase assay and brine shrimp lethality bioassay. *Alpinia nigra* extracts showed positive result on antioxidant and antityrosinase activities. We found that extract of *A. nigra*'s leaf has the most effective activity of antioxidant and antityrosinase among other parts of this plant. The ethanol and aqueous extracts from the leaf of *A. nigra* at the concentration of 125 µg/mL showed % inhibition for free radical scavenging as 94.97% and 93.35%, respectively. The IC_{50} values of antioxidant were 39.83±16.21 and 46.33±15.22 µg/mL, respectively. In addition, ethanol extract of the leaf from *A. nigra* at the concentration of 1,000 µg/mL produced 92.61% inhibition of mushroom tyrosinase activity, whereas aqueous extract of *A. nigra*'s leaf at the same concentration produced 74.47% inhibition. The IC_{50} of antityrosinase activities were 142.81±13.32 and 406.88±66.43 µg/mL for ethanol and aqueous extracts, respectively. Moreover, the brine shrimp lethality bioassay showed that all extracts were non-toxic (LC_{50}>1,000 µg/mL). In conclusion, the ethanol extract of *A. nigra*'s leaf may be beneficial and provide the novel and safe source for antioxidant and whitening agent.

Keywords: *Alpinia nigra*, antioxidant, antityrosinase, toxicity

Alpha-mangostin phase inversion induced *in situ* forming gel

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Abstract. Alpha-mangostin (aMG) phase inversion induced *in situ* forming gel (ISG) was prepared by dissolving matrix formers including palmitic acid (P) and lauric acid (L) in dimethyl sulfoxide (DMSO) and *N*-methyl pyrrolidone (NMP). The pH and density values were in range of 4.42-6.22 and 0.9731-1.0943 $g.cm^{-3}$, respectively. The prepared formulas have low viscosity and high injectability which were suitable for injection. The gel formation behavior was affected by type of fatty acid and solvent. P dissolved in DMSO showed a rapid transformation into matrix at initial time. The extended release of aMG was attained in P-based ISG. aMG phase inversion induced ISG improved microbial inhibition.

Keywords: alpha-mangostin, *in situ* forming gel, phase inversion

Liposomal approach to the development of Thai herbal products: hydrophilic- and lipophilic-extractions

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Abstract. Liposome is a drug delivery system utilized to overcome the limitation of various drugs. Water solubility of active compounds is an important factor affecting the design and development of pharmaceutical products. The objective of this study was to develop a liposomal delivery system of Thai herbal products. The specific water solubility was the primary factor indicating the selection of a model Thai herbal plant. The model plants for lipophilic extractions (turmeric and finger root) and hydrophilic extractions (tomato and aloe) were selected. Liposome formulations were prepared by sonication. Lipophilic- and hydrophilic extracts were incorporated in the rigid- and elastic-liposomes. The physicochemical characteristics (e.g., vesicle size, size distribution and zeta potential) and stability of the liposome formulations were evaluated. The average sizes and size distributions of the liposomes were 180 to 1170 nm and 0.3 to 0.9, respectively, depending on the type of liposomes. The liposomes displayed positive or negative charges. The finding indicated that the formulation factors and method of preparation were not only the causal factors, but the intrinsic properties of plant extracts also affected the physicochemical characteristics and stability. These results suggested that the rigid liposome and the anionic elastic liposome might be alternative nanovescicles for the delivery of Thai herbal products.

Keywords: nanovesicles, turmeric, finger root, tomato, aloe, plant extract