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1-5 pm
S16 Lecture Theatre 31

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Visiting Professor at the National University of Singapore and at the Pharmacy Department of Ljubljana in Slovenia; Guest Professor at the Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok, Thailand.

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Senior Manager/ Human Protection Administrator
NUS Institutional Review Board

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PLENARY PRESENTATION 1

Transdermal Iontophoresis of Apomorphine – from in-vitro Modelling to the Treatment of Parkinson Patients

Hans E. Junginger
Visiting Professor at the Dept. of Pharmaceutics, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok, Thailand and at the National University of Singapore

Biography

H.E. Junginger, Ph.D. was Professor of Pharmaceutics and Head of the Division of Pharmaceutical Technology at the Leiden/Amsterdam Center for Drug Research, Leiden University, The Netherlands until 1 February 2004 when he went with early retirement
Since 1 January 2004 he is a guest professor at the Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok, in Thailand and since 2005 also a Visiting Professor at the National University of Singapore and at the Pharmacy Department of Ljubljana in Slovenia.

He qualified as a pharmacist in 1967, at the University of Munich, Germany. In 1971, he obtained his Ph.D. degree in Pharmaceutical Chemistry at the University of Saarbrűcken, Germany. 1972 –1980 he worked as a researcher at the Technical University of Braunschweig, Germany to obtain his qualification as professor in Pharmaceutical Technology. Since 1980 – 2004 he was the head of the Department of Pharmaceutical Technology at the Leiden/Amsterdam Center for Drug Research, The Netherlands.

He has published more than 280 articles and 35 book chapters. He is the (co)inventor of 8 patents.

His main research areas included the development of novel controlled drug delivery systems (especially for peptide drugs) for the (trans)dermal and peroral routes, utilizing new (bioadhesive) polymers. Especially multifunctional polymers as polyacrylates and chitosan derivatives have been identified to be safe and non-toxic penetration enhancers for hydrophilic drugs. Furthermore, they are excellent delivery systems for the nasal, pulmonary and oral route for peptides, protein and (DNA) vaccines. Combining superporous hydrogels or other expanding tablet systems with those multifunctional polymers make the oral absorption of peptides feasible.

Until now 52 Ph.D. students have graduated under his supervision and 25 post-docs from all over the world have joined his department in Leiden.

He was 1986-1990 president of the International Association for Pharmaceutical Technology (APV) and 1994-5 president of the Controlled Release Society.
He was the Scientific Secretary of the International Pharmaceutical Federation FIP (1995 – 2003) and as such member of the Executive Committee of FIP.
He has received several major awards and three honorary doctorates (Ghent, Belgium in 1995, Potchefstroom University in South Africa in 2003 and London University, UK in 2004).

He is a frequently invited speaker at international conferences and a consultant to international pharmaceutical industries.
He loves traveling and as result of this his nickname is "Flying Dutchman". When at home he loves to play piano and to read criminal stories.

Abstract

Transdermal Iontophoresis of Apomorphine – from in-vitro Modelling to the Treatment of Parkinson Patients

Hans E. Junginger
Visiting Professor at the Dept. of Pharmaceutics, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok, Thailand and at the National University of Singapore

Optimal drug delivery will remain a challenge also in the 21st century. Optimal drug delivery means that a drug is delivered in the right dose at the right time at the right place without inducing toxic side effects. Although a lot of progress has been made to achieve these goals, drug delivery is still far away from being optimal at the current time both with respect to reproducibility and predictability not to mention at all the desirable "drug delivery on demand" which means optimised drug delivery depending on the state of disease.
However, drug monitoring of the achieved drug levels is still far away from reality. Transdermal iontophoresis, i.e. the electrically driven transport of charged drug molecules by means of a mild current across human skin may be able to overcome this problem for special diseases. Iontophoresis of apomorphine, a potent anti-Parkinson disease drug with poor oral bioavailability is able to achieve therapeutic drug levels in Parkinson patients. Disease parameters such as rigor (stiffness) and tremor (trembling) can be monitored by suitable chips. Feedback with those signals with the computer of the iontophoretic delivery system may result in optimal disease treatment by drug input on demand avoiding toxic drug levels.
Although a lot of research has been done to develop therapeutic transdermal systems for therapeutic use, the achievements so-far have been disappointing. In 2006 the first simple iontophoretic systems with lidocaine as drug for local anaesthesia was finally approved by FDA. ALZA also has now FDA approval for commercialization of an iontophoretic fentanyl patch.

Some references
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PLENARY PRESENATION 2

Ethics in Research using Human Participants

Chan Tuck Wai
Certified IRB Professional (CIP USA)
Bachelor of Science (Pharmacy), Master of Business Administration
Senior Manager & Secretariat
National University of Singapore - Institutional Review Board, Singapore

Biography

Mr. Chan Tuck Wai is the Senior Manager and Secretariat of NUS Institutional Review Board (NUS-IRB). He is also the appointed Human Protections Administrator of the National University of Singapore.

Mr. Chan is a Registered Pharmacist in Singapore, with Masters of Business Administration from Golden Gate University, San Francisco. He is also the only Certified IRB Professional (CIP) by the Applied Research Ethics National Association (ARENA)**,** USA in ASIA, and has presented a paper during the 2004 and 2006 annual conferences. Mr. Chan was appointed as temporary adviser to WHO and invited to attend Conference on Ethical Aspects of International Collaborative Research and Health Ethics, 2005.

Mr. Chan was the Head of Medical Affairs, National University Hospital and was instrumental in the setting up of the first university IRB - NUS-IRB, and the first Nursing and Paramedical IRB (NUH NP-IRB) in Singapore.

Prior to this appointment in NUH, Mr. Chan held the position of Senior Manager, Asia Pacific Regional Regulatory Affairs and Medical Affairs with Aventis-Behring (Asia Pacific), and handled both drug regulatory affairs and multi-centre clinical trials in the Asia Pacific region.

Mr. Chan was the Editor-in-Chief of HealthWorld Asia, a health magazine published in USA, and the Editor of bulletins of the Pharmaceutical Society of Singapore and NUS Pharmaceutical Society.

Abstract

Ethics in Research using Human Participants

We are going through a time of profound change in our understanding of the ethics in research using human participants.

From the time immediately after World War II until the late 1990s, there was a gradually developing consensus about the key ethical principles that should underlie the research endeavor.

Historical events in research misconduct and violation of ethical principles stood out as symbolic of this consensus. The German Nuremberg War Crimes Trial and the Japanese War Crimes following World War II, brought to public view the ways some scientists had used human as subjects in gruesome experiments. In the 1950s and 1970s, the Tuskegee Syphilis Study involved the withholding of known effective treatment for syphilis from African-American participants who were infected.

Events like these forced the reexamination of ethical standards and the gradual development of a consensus that potential human subjects needed to be protected from being used as 'guinea pigs' in scientific research.

In this talk, the speaker will help researchers identify conflicts of interest, plan research, recruit participants, working with special populations, updating issues relating to institutional review boards, and manage matters of informed consent, privacy, confidentiality and maintain the participants' trust and safety.

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PODIUM PRESENTATIONS

Anticoagulant Effects of Extracts of *Ardisia elliptica*

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Introduction

According to WHO, cardiovascular diseases are one of the major killers of the global population[1]. Warfarin is an anticoagulant drug commonly used in treatment of cardiovascular diseases, but it has a narrow therapeutic window. Thus it is of utmost importance to continue to search for novel and safer anticoagulants. *Ardisia elliptica* is traditionally used to treat chest pains[2], and has been reported to have anticoagulant properties[3]. With the increasing interest in the discovery of drugs from plants, the objective of this project is to further investigate *A. elliptica*'s potential anticoagulant properties.

Experimental

Fresh leaves of *A. elliptica* were collected, ground by a blender, and subjected to soxhlet extraction for 6 hours using solvents of different polarity. Hexane, 70% methanol and water were employed for the extraction of compounds.

The 70 % methanol extract was further separated by a bioassay guided fractionation, and the compounds were analysed by a Shimadzu GC-MS with a Shimadzu DB-5 MS column of internal diameter 0.25mm, length 30.0m and film thickness 0.25µm. Plasma coagulation assays were performed to elucidate the effects of the extracts on the prothrombin time (PT) and activated partial thromboplastin time (aPTT) using a Blood Coagulation Analyzer CA-500 Series (Sysmex, Kobe, Japan).

Results and discussion

A comparison of the anticoagulation effects of the hexane, 70% methanol and water extracts of *A. elliptica* is shown in Table 1.

Table 1 : A comparison of anticoagulant activities of different extracts of *A. elliptica*.

	Concentration / mgml^{-1}	PT / s	aPTT / s
0.17 % DMSO in PBS (control)		15.10 ± 0.36 13.83 ± 0.06	38.57 ± 0.45 40.97 ± 0.15
Hexane extract	0.39	*	*
70 % MeOH extract	3.14	23.03 ± 3.80 *	20.93 ± 1.67 *
Water extract	4.72	NC *	NC *
Heparin	0.004	15.60 ± 0.26	81.03 ± 7.40*

* $p<0.05$; NC: no coagulation observed

As can be seen from Table 1, the hexane extract was the least soluble in 0.17% (v/v) DMSO in PBS and exerted only slight effects on the PT and aPTT. The 70% methanol extract was more soluble, and was observed to increase the PT by 1.53 times. However the aPTT was decreased 1.84 times by the 70% methanol. The most potent activity was exerted by the water extract, which was able to completely inhibit plasma coagulation.

The dose response of the effects of PT and aPTT by the 70% methanol and water extracts are shown in Fig. 1.

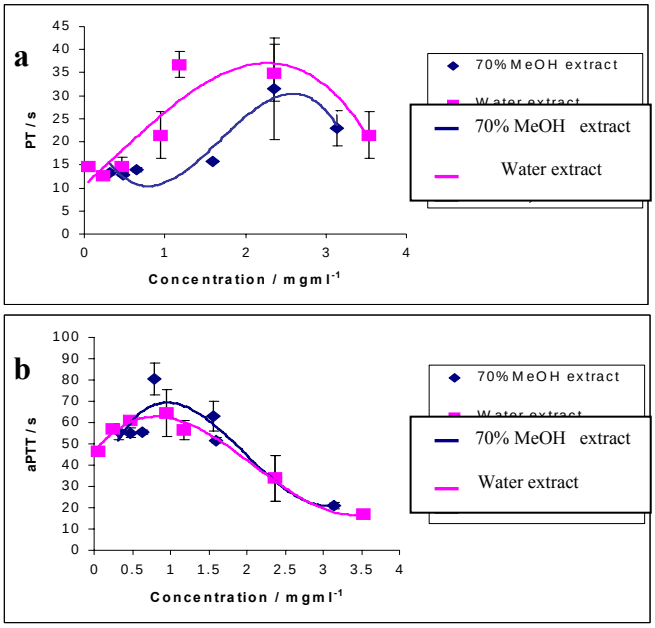

Figure 1 : Effects of extracts on a) PT and b) aPTT.

A concentration of 2.4mgml^{-1} and 2.3mgml^{-1} of the 70% methanol and water extracts respectively were found to exert maximal effects on prolonging the PT (Fig. 1a). Total inhibition of plasma coagulation by the extrinsic pathway was seen above 3.5 mgml^{-1} of the water extract. Maximal prolongation of the aPTT was observed at 0.8mgml^{-1} and 0.9mgml^{-1} for the 70% methanol extract and the water extracts respectively (Fig. 1b). Similarly, no coagulation by the intrinsic pathway was observed above 3.5 mgml^{-1} for the water extract.

As an attempt to further elucidate the compounds responsible for the anticoagulant activities, a bioassay guided fractionation was performed on the 70% methanol extract. A total of 13 fractions were collected, and the seventh fraction collected exhibited the most potent anticoagulant activity. This fraction was able to prolong the PT by 1.3 times and the aPTT by 3.8 times compared to the control.

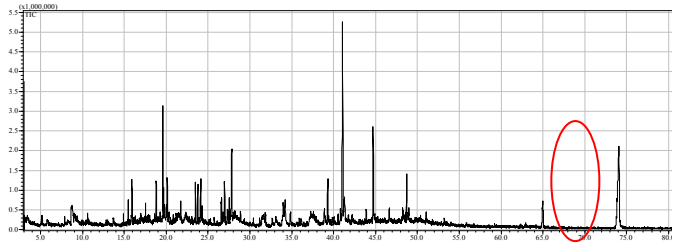

Figure 2 : A gas chromatogram of Fraction 7.

A comparison of the chromatograms produced by GC-MS of the different fractions showed that a compound unique only to Fraction 7 was eluted at 74.077 min (Fig.2). Most compounds eluted in this fraction include fatty acids such as myristic acid and palmitic acid.

Conclusion

The 70% methanol and water extracts of *A. elliptica* were found to exhibit potent anticoagulant activities. Further studies need to be conducted to elucidate the compounds responsible for the anticoagulant activities of *A. elliptica*.

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I would like to thank my supervisors, Dr Koh Hwee Ling and A/P Tan Chay Hoon for their advice, as well as my lab colleagues for their support.

USING ALGINATE COMPOSITE AS WALL MATERIAL TO ACHIEVE MICROENCAPSULATION WITH HIGH OIL LOADING

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Introduction

Fish oils have been found to have numerous benefits to human health[1,2]. This was due to the high amounts of ω-3 polyunsaturated fatty acids, mainly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) present within them. However, fish oils are prone to oxidative degradation and rancidity due to their high levels of unsaturation. Microencapsulation of fish oil has been carried out in various studies to improve stability and ease of handling[3,4]. In this study, alginate-containing wall material was used for production of microspheres with high loading by spray drying.

Experimental

Emulsions were prepared according to the formulae in Table 1 and spray dried using a pilot-scale spray dryer (Mobile Minor, Niro, Denmark, Germany).

Table 1. Composition of different microsphere formulations studied.

Code	Component (g)			
	Modified starch	Low G alginate	High G alginate	Fish oil
C	225			337.5
LB1	210	15		337.5
LB5	150	75		337.5
LB10	75	150		337.5
LBB1	210		15	337.5
LBB5	150		75	337.5
LBB10	75		150	337.5

Microspheres were visually examined by scanning electron microscopy (JEOL, JSM-5200, Japan). Size and roundness determination were carried out using light microscopy (BX61TRF, Olympus) interfaced with an image analysis system (MicroImage™, Olympus, Japan). Microencapsulation efficiency (ME) was calculated using the difference between total and surface oil of a known weight of microspheres before and after Soxhlet extraction (B-811, Büchi Labortechnik AG, Switzerland).

EPA and DHA content on storage were determined after incubation of accurately weighed microspheres at 40 ^{0}C and 70 % R.H. At specific time intervals, samples were extracted with hexane and quantitation carried out by gas chromatography (HP5890II, Agilent Technologies, USA).

Results and discussion

Microspheres produced were generally spherical with no surface cracks. However, there was a difference in their degree of surface indentation. Microspheres produced with a greater proportion of alginate seemed to have fewer surface indentations. This was more so when Manucol LB® was used. Microspheres made using purely Capsul® as wall material appeared the most dimpled (Fig 1).

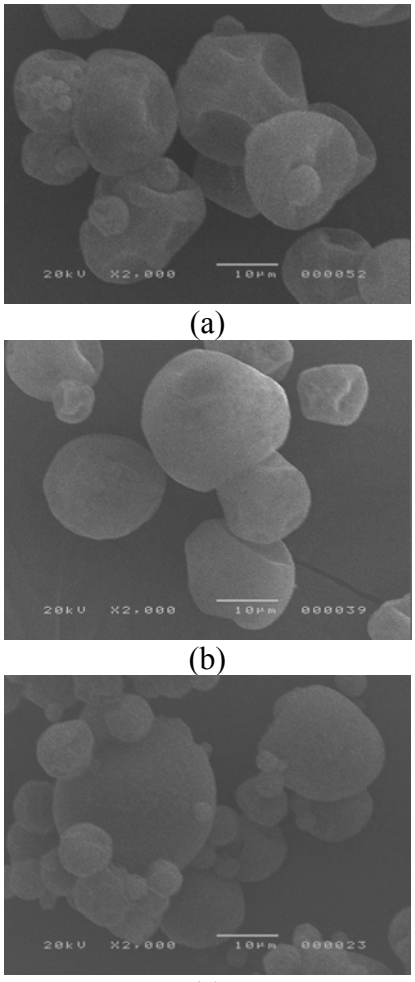

Figure 1. SEM of spray-dried microspheres using formulation (a) C, (b) LBB10 and (c) LB10.

The presence of surface indentations was due to particle shrinkage during the droplet drying stage of spray drying[5]. It could thus be deduced that microspheres containing alginate as wall material were more resistant to shrinkage during drying. This could also imply that the addition of alginate resulted in a stronger microsphere matrix being formed, although further studies were needed to confirm this.

Microspheres produced from the various formulations were generally similar in size ($p > 0.05$). Roundness increased as alginate proportion increased. Yield and ME were also correspondingly higher. These differences were more significant with Manucol LB®.

The effect of alginate substitution on roundness was most likely due to the phenomenon of shrinkage as mentioned earlier. This in turn allowed more oil to be encapsulated within the microspheres, as particle shrinkage would likely have resulted in oil being squeezed out from the microsphere cores. The higher yields obtained with formulations LB5, LB10, LBB5 and LBB10 could have been due to the presence of lower amounts of surface oil on the microspheres, reducing their adherence to the inner walls of the spray dryer and allowing more product to be collected at the end of the process stream.

Table 2. Mean microsphere size, roundness, yield and ME of microspheres prepared using the different formulations.

Formula	Size (μm)	[a]Round-ness	Yield (%)	ME (%)
C	18.9(*0.4*)	1.13	47.8(*7.5*)	57.4(*2.9*)
LB1	18.6(*0.3*)	1.12	51.3(*5.8*)	59.4(*2.0*)
LB5	19.1(*0.2*)	1.10	65.5(*4.3*)	67.3(*2.7*)
LB10	19.8(*0.3*)	1.08	72.6(*4.5*)	76.6(*2.1*)
LBB1	18.8(*0.3*)	1.12	49.2(*5.2*)	60.8(*3.4*)
LBB5	19.0(*0.4*)	1.12	58.7(*4.9*)	66.2(*2.8*)
LBB10	19.2(*0.3*)	1.10	68.6(*4.7*)	72.2(*1.1*)

[a]Standard deviation < 0.001

EPA and DHA contents on storage were monitored to assess the ability of microspheres to preserve the integrity of the encapsulated oil. It was observed that for all the microspheres produced, the greatest decrease in EPA and DHA contents occurred over the first 3 days of storage. This was likely due to surface oil degradation. The decrease became more gradual as oxygen penetration into the microspheres was impeded by the presence of the wall matrix, finally reaching a plateau at around Day 42. Overall, formulation LB10 gave the highest retention of EPA and DHA.

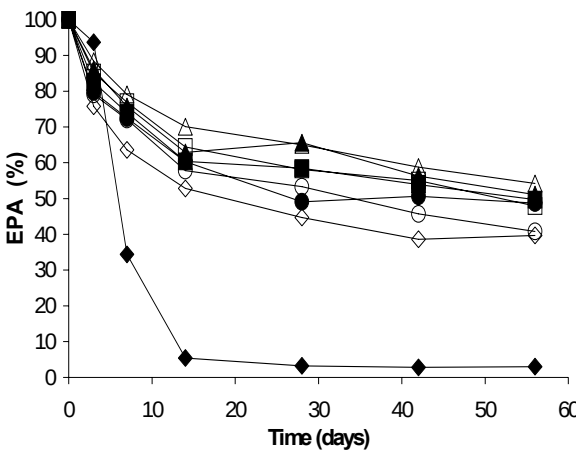

Figure 2. EPA content on storage for ♦unencapsulated oil, ◊C, ○LB1, □LB5, ΔLB10, ●LBB1, ■LBB5, ▲LBB10. (DHA curve similar, not shown)

Conclusion

Alginate-composite microspheres were successfully produced by spray drying, with high oil loadings achieved. The use of alginate allowed rounder microspheres with greater oil holding capacities to be formed. Microencapsulation of fish oil brought about lower loss on storage compared to unencapsulated oil.

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QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIP (QSAR) OF INDOLOACETAMIDES AS INHIBITORS OF HUMAN ISOPRENYLCYSTEINE CARBOXYL METHYLTRANSFERASE

Jo-Lene Leow*[a], Rudi Baron[b], Patrick J Casey[b], Mei-Lin Go[a]

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Introduction

RAS and many other oncogenic proteins contain a C-terminal CAAX motif which directs the protein through post-translational modifications important for their localization and function. This processing is initiated by prenylation of the cysteine residue, followed by C-terminal proteolysis and carboxymethylation. Although inhibitors that have been designed to target the prenylation step, particularly farnesyltransferase-catalyzed prenylation, are now in advanced stage clinical trials, their utility and efficacy seem to be limited. Recent findings indicate that inhibition post-prenylation processing steps, especially the isoprenylcysteine carboxyl methyl-transferase (ICMT), provide a more profound effect on oncogenesis. An indole-based selective inhibitor of ICMT, cysmethynil (IC50 = 2.4μM), was identified through the screening of a diverse chemical library.

Experimental

A QSAR is developed for the ICMT inhibitory activities of a series of indoloacetamides (n = 72) that are structurally related to cysmethynil. Multivariate analytical tools (principal component analysis, PCA, and projection to latent structures, PLS), multi-linear regression (MLR) and comparative molecular field analysis (CoMFA) are used to develop a suitably predictive model for the purpose of optimizing and identifying members with more potent inhibitory activity.

Results and discussion

A significant 3 component model (r^2 =0.72, q^2 = 0.54) is obtained from PCA, where the 1st component receives input from size (volume, area, molar refractivity) and lipophilic (ClogP) parameters. The best PLS model has an r^2 of 0.72 and q^2 of 0.60. The coefficient plot obtained indicates that the most significant parameters are polar surface area (PSA) and polar volume (PV), followed by the lipophilicity of phenyl ring (π_{Ph}) and size and lipophilicity of N substituents.

The stepwise multiple linear regression (MLR) equation derived has four descriptors (PSA, PV, Sterimol parameter B1 of the substituted phenyl ring and π_{Ph}).

The best CoMFA model has a cross validated q^2 of 0.646 (7 principal components) with a standard error of prediction (SEP) of 0.342, and a non cross-validated r^2 of 0.868 with standard error of estimate (SEE) of 0.209. The steric and electrostatic field contributions are 68% and 32% respectively, indicating a greater steric influence on activity. Visualization of the steric contours indicate that there are two optimal lengths of the N substituent and a less bulky phenyl ring is for good activity.

Conclusion

The resulting model shows that good activity is determined largely by the characteristics of the substituent attached to the indole nitrogen, which should be a lipophilic residue with fairly wide dimensions. In contrast, the substituted phenyl ring attached to the indole ring must be of limited dimensions and lipophilicity.

Evaluation of Triptolide's molecular effects on the cell viability and *in vitro* invasiveness of breast cancer cells

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Introduction Overexpression of urokinase-type plasminogen activator (uPA) is implicated in breast cancer metastasis. It is thus important to identify uPA inhibitors as potential therapeutic options for the treatment of metastatic breast cancer. Triptolide, an active component purified from a Chinese herb, exerts potent anticancer activity against several tumor cells, and is reported to inhibit PI3K activity. Akt/PKB, an important downstream target of PI3K, is a critical regulator of uPA; inhibition of phosphorylated Akt (p-Akt) results in suppression of uPA expression. In view of triptolide's inhibition of PI3K, we therefore hypothesized that triptolide would inhibit Akt activation of breast cancer cells, thereby decreasing their uPA expression and reducing their invasiveness.

Experimental Methods Triptolide's cytotoxic and apoptotic effects on MDA-MB-231, MCF-7-her-2, MCF-7 and JIMT-1 cells were determined using MTT and DNA fragmentation assay, respectively. JIMT-1 is a Her-2/neu-overexpressing cell line that is resistant to trastuzumab. MTT assay was performed to investigate whether triptolide could sensitize JIMT-1 to trastuzumab. Triptolide's effects on MDA-MB-231 and JIMT-1's p-Akt levels were studied using Western Blot. We also investigated triptolide's effects on MDA-MB-231's uPA secretion and *in vitro* invasiveness by ELISA and matrigel invasion assay, respectively.

Results and discussion Triptolide exhibited potent cytotoxic effects on all four breast cancer cell lines, with IC_{50} values ranging from 1.57 to 6.16ng/ml.DNA fragmentation was also observed with triptolide-treated cells, suggesting that triptolide induced apoptosis in breast cancer cells. Furthermore, triptolide decreased MDA-MB-231's p-Akt levels, uPA secretion and matrigel invasion. Unexpectedly however, triptolide increased p-Akt level in JIMT-1, and failed to sensitize JIMT-1 cells to trastuzumab. Our results suggest that triptolide's inhibition of p-Akt might explain its induction of apoptosis, reduction of uPA secretion and decrease of matrigel invasion in MDA-MB-231. Nevertheless, the role of Akt in triptolide's cytotoxic effect against JIMT-1 remains unclear. It is possible that triptolide's molecular action is cell-type specific, and therefore triggers apoptosis in JIMT-1 by regulating an Akt-independent pathway. Another possible explanation is that the initial Akt induction observed might be instrumental to triptolide's cytotoxic effect in JIMT-1.By exploiting JIMT-1 cells' initial dependence on Akt phosphorylation as an anti-apoptotic route, triptolide renders them more susceptible to its subsequent inhibition of p-Akt, leading eventually to apoptosis.

Conclusion Overall, these findings provide additional mechanistic insights into triptolide's anticancer activity and support its role in metastatic breast cancer treatment.

CALCIUM CARBONATE NANOPARTICLES AS AN ANTI-TACK AGENT IN FINE PARTICLE COATING

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Introduction

This study employed precision coating (Precision Coater, GEA-Aeromatic Fielder, UK), a bottom spray fluid bed process, to form coated microparticles by spray coating a polymeric material onto fine lactose particles.

The main pharmaceutical advantage of coated microparticles is the ability to be processed into tablets without having as much damage to the functional coats as compared to larger coated multiparticulates which have to be processed into capsule dosage forms, a comparatively less cost effective method.[1]

Among the different methods of forming coated microparticles, bottom spray fluid bed coating is superior in the ability to scale-up. Highly functional multi-layer microcapsules from fine particles as small as 10 µm have been produced by this method.[2] The drug may be presented as the core particles or coated in the same or different layers as the polymer around inert core particles. However, due to the high tendency of agglomeration and poor flow of fine particles, the spray rate is compromised making the coating process very time consuming. Hence, there is a need to improve the fine particle coating process.

In this study, the use of calcium carbonate nanoparticles (CCn) as a surface modifying agent, in an attempt to reduce the agglomeration during fine particle coating was investigated.

Experimental

600 g of lactose particles (Tablettose 80, Meggle Pharma, Germany), pre-sieved to obtain a median size of 166 ± 1.8 µm, were first coated with 24 g of CCn suspension at a spray rate of 4 g/min followed by 300 g of 10 % w/w hypromellose aqueous solution (HPMC: Methocel E3, Dow Chemical, USA) at a spray rate of 10 g/min (Figure 1). Inlet temperature of 60 °C, airflow rate of 20 m^3/h and atomizing pressure of 2 bar were used as the standard conditions for coating.

For surface modification, suspensions containing 0.6, 1.2, 1.8, 2.4 or 3.0 g of CCn in deionized water were used and are presented as a percentage of the lactose load. Deionized water without CCn was used as the control i.e. untreated group.

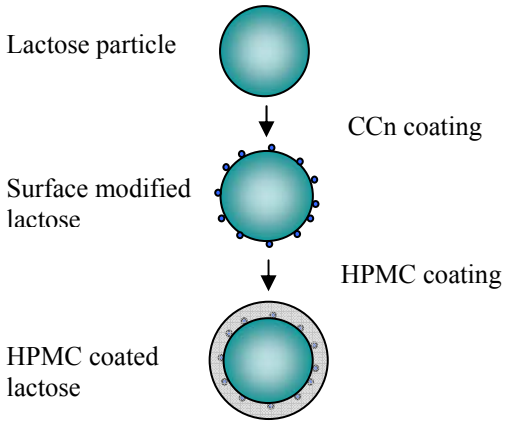


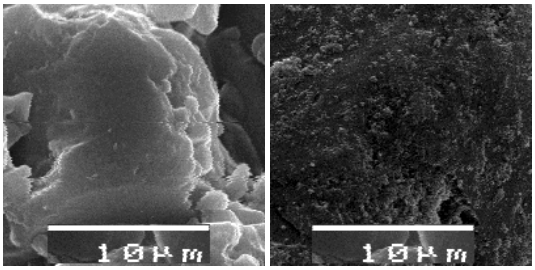
Figure 1. Flow diagram of the coating process for the coating of lactose microparticles.

Surface-modified lactose microparticles were tested for flow properties using angle of repose (Powder tester, Hosokawa Micron Corporation, Japan), their morphology examined using a scanning electron microscope (JSM-5200, Jeol, Japan), and surface roughness described using the arithmetic mean height, Ra, obtained from a scanning probe microscope (SPM-9500J, Shimadzu, Japan) under dynamic mode over scan area of 10 x 10 µm.

Size of HPMC coated lactose microparticles was determined using the dry powder module of a laser diffraction particle sizer (LS230, Coulter Corporation, USA).

Results and Discussion

Lactose surfaces were successfully coated with CCn by precision coating as seen in SEM micrographs in Figure 2. Being relatively smaller in size than lactose particles, CCn was distributed over the lactose surfaces but did not cause significant change to the surface roughness.



Untreated lactose surface (Ra = 460 ± 199)

Lactose surface with 0.5 %w/w calcium carbonate nanoparticles (Ra = 421 ± 175)

Figure 2. Scanning electron micrographs of lactose surfaces and their surface roughness.

The coated lactose showed improved flow as seen in the smaller angle of repose (Figure 3). As the CCn were in the nano-sized range, it is postulated that their adhesion to the lactose was by electrostatic and/or van der Waal's forces. Hence the improved flow could be due to the CCn acting as nano-sized ball bearings, providing dry lubrication to the particles. This effect seemed to approach a plateau at around 0.4 to 0.5 % w/w of CCn, probably due to saturation of the lactose surfaces with CCn.

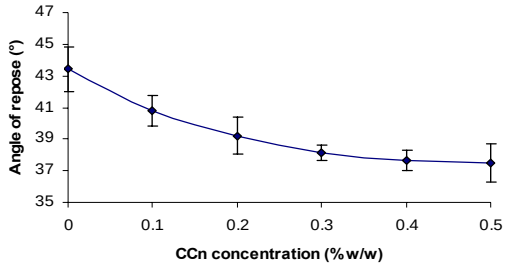


Figure 3. Angle of repose of surface-modified lactose particles with increasing concentration of CCn used.

Compared with untreated lactose, the CCn-coated lactose showed lower extent of agglomeration in subsequent coating with HPMC, particularly in the d10 (maximum size of 10 % of the smallest particles) (Figure 4). It did not significantly alter the d90 (maximum size of 90 % of the smallest particles) and only caused a significant reduction in the d50 (median size) at 0.3 % w/w of CCn. This indicated that CCn coating exhibited anti-tack behaviour especially for particles smaller than ~100 µm in size.

As seen in Figure 4, the d10 value decreased to a minimum at 0.3 % w/w and approached a plateau. This trend correlated significantly ($p = 0.005$) with the angle of repose, having an R^2 value of 0.86. This showed that flow property of core particles was a major factor affecting agglomeration of fine particles.

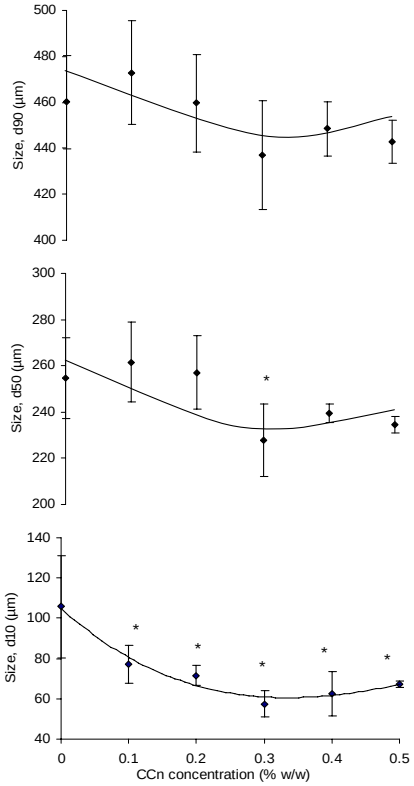


Figure 4. Particle size of HPMC coated CCn surface-modified lactose particles.
* significant difference ($p < 0.05$) compared to size of untreated lactose particles.

Conclusions

CCn adhered to the surface of core particles, where it served as a dry lubricant. This probably resulted in its anti-tack action in the HPMC coating of fine particles. The concentration of CCn used has to be optimal to provide the best benefit for fine particle coating.

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POSTER PRESENTATIONS

DESIGN AND SYNTHESIS OF SUBSTITUTED 2,6-DIAMINO-5-AZAPURINES WITH POTENTIAL ANTIFOLATE ACTIVITY

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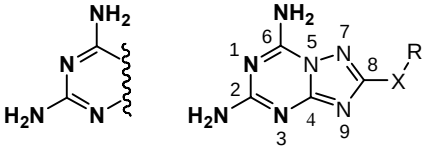
Introduction

The diamino heterocyclic structure **1** forms an essential part of the pharmacophore in the molecules of dihydrofolate reductase (DHFR) inhibitors such as methotrexate, trimethoprim, cycloguanil and trimetrexate [1]. The structure **1** often represents a part of the fused ring system. With our interest towards 5-azapurine (1,2,4-triazolo[1,5-*a*][1,3,5]triazine) heterocyclic nucleus [2], we report herein the synthesis of 2,6-diamino-5-azapurines **2**, which may possess DHFR inhibitory activity. In order to mimic the distal part of the "nonclassical" DHFR inhibitors, the lipophilic moiety R (phenyl or 3,4,5-trimethoxyphenyl) was introduced into the side chain of the compounds **2**. The 5-azapurine nucleus is ether linked to the lipophilic aromatic part directly or has a spacer linker (X) introduced between the heterocyclic skeleton and R.



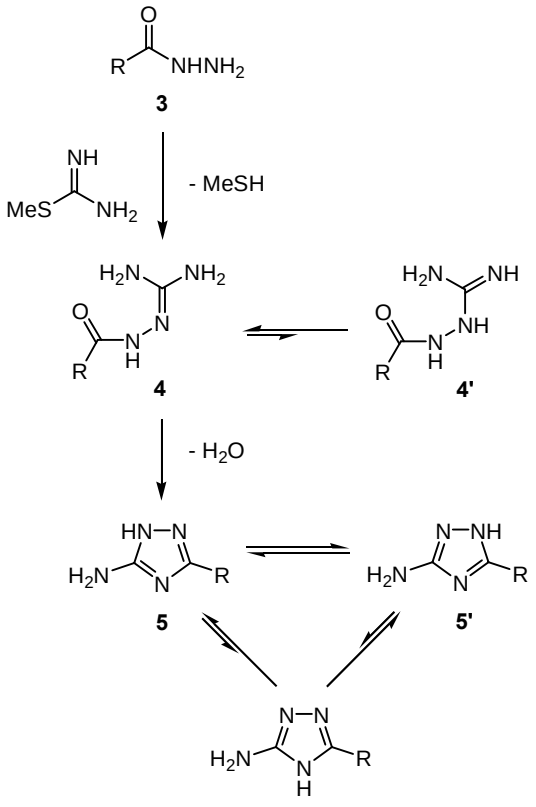
1 **2**

Results and discussion

Two methods for the preparation of the key intermediates 3(5)-amino-1,2,4-triazoles were used.

Method 1. The reaction of hydrazides **3** with *S*-methylisothiourea provided *N*-acylaminoguanidines **4**. Benzamidoguanidine (**4a**) was further cyclized on heating to the corresponding triazole **5a**. The compound **4b** was readily cyclyzed to **5b** at the reaction conditions and therefore was not isolated.

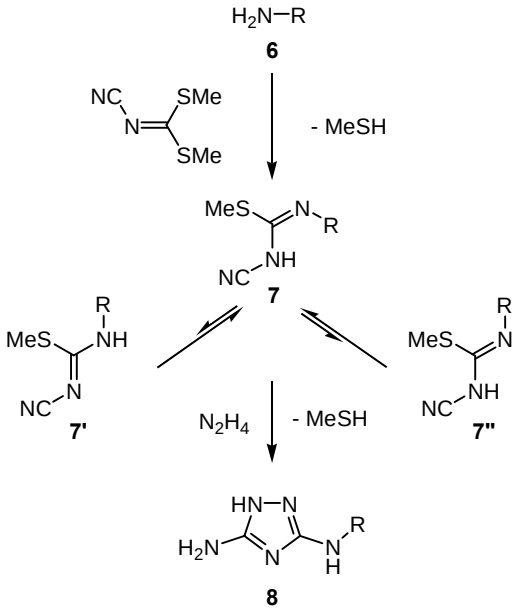
The investigation of tautomeric preferences for the intermediates **4** and **5** showed that benzamidoguanidine (**4a**) existed in the form **4**, but not **4'**. In the triazoles equilibrium, 5-amino form **5** was predominant, 3-amino form **5'** was minor ($K_{T(5/5')}$ = 9 (**a**) and 4 (**b**); ΔG_{298} = -5.4 (**a**) and -3.4 (**b**) kJ/mol), whereas **5''** was not present to any measurable extent.



3 **4** **4'** **5** **5'** **5"**

3-5: R = Ph (**a**), Bn (**b**)

Method 2. The 3,5-diamino-1,2,4-triazoles **8** were prepared using partial aminolysis of *N*-cyanodithiocarbonimidate with amines **6** followed by cyclization of the *N*-substituted *N'*-cyano-*S*-methylisothioureas **7** with hydrazine.

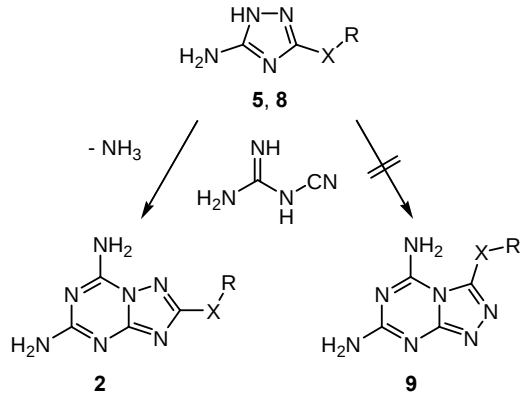


6-8: R = Ph (**a**), Bn (**b**), CH_2CH_2Ph (**c**), $C_6H_2(OMe)_3$-3,4,5 (**d**), $CH_2C_6H_2(OMe)_3$-3,4,5 (**e**)

It was found that isothioureas **7** existed in the form of tautomer **7**, but not **7'**; *E*-configuration **7** was preferred over *Z*-configuration **7''**.

The (3+3) heterocyclization of 3(5)-amino-1,2,4-triazoles **5** and **8** using cyanoguanidine as C-N-C fragment introducing reagent was successfully applied for the synthesis of 5-azapurines **2**. The reaction proceeded with liberation of ammonia and required high temperature (reflux in DMF) or acidic catalysis (HCl) in aqueous medium.

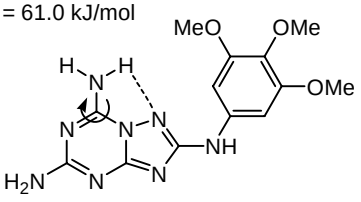
The heterocyclization was found to be regioselective and only 5-azapurines **2** were isolated. The theoretically possible formation of 1,2,4-triazolo[4,3-*a*][1,3,5]triazines **9** was excluded based on NMR spectral data.



2: R = Ph (**a**-**e**), $C_6H_2(OMe)_3$-3,4,5 (**f**,**g**);
X = - (**a**), CH_2 (**b**), NH (**c**,**f**), $NHCH_2$ (**d**,**g**), $NHCH_2CH_2$ (**e**)

The weak intramolecular hydrogen bonding observed in compounds **2** led to the hindered rotation across the C(6)-NH_2 bond. The free energy of activation ($G^{\ddagger}$) of the rotation at the coalescence temperature was estimated for **2f**.

$G^{\ddagger}_{301}$ = 61.0 kJ/mol



2f

Conclusion

A new method for the preparation of the substituted 2,6-diamino-5-azapurines as potential DHFR inhibitors was successfully developed.

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Infrared-based protocol for the identification and categorization of ginseng and its products

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Introduction

Quality assurance of ginseng is important since many ginseng products sold today are in various formulations, and it is difficult to identify them by morphological means such as physical appearance, smell, or even taste. Different chemical constituents in herbs like ginseng tend to exhibit characteristic infrared fingerprints. The objective of this study was to establish a protocol based on infrared wavelengths and chemometrics to perform rapid quality surveillance of traditional Chinese medicines (TCMs), with ginseng as an example.

Experimental

Tests were carried out on three grades of ginseng root powders from Hong Kong and on three commercial products. The purpose of these tests was to confirm if the proposed protocol could be used to identify unknown samples of ginseng and discriminate commercial ginseng products. The identities of the types of ginsengs were not made known during the time of purchase, but the test results were subsequently verified by Mr. Gary Pang, district manager of Hockhua Ginseng Birdnest Trading Enterprises, a major TCM retail organization in Singapore.

Results and discussion

We have established a simple and rapid protocol based on infrared wavelengths and principal component analysis for the identification and categorization of ginseng, and called it the 2-6PC rule. The advantage of this protocol is that it is able to provide rapid identification of natural products since it avoids tedious extraction or purification procedures. Our results show that this protocol was not only able to discriminate raw ginseng roots, but also different types of ginsengs in three commercial ginseng products.

Conclusion

The potential of the novel 2-6PC rule as a rapid quality surveillance tool in the authentication of ginseng and its products is definitely appealing.

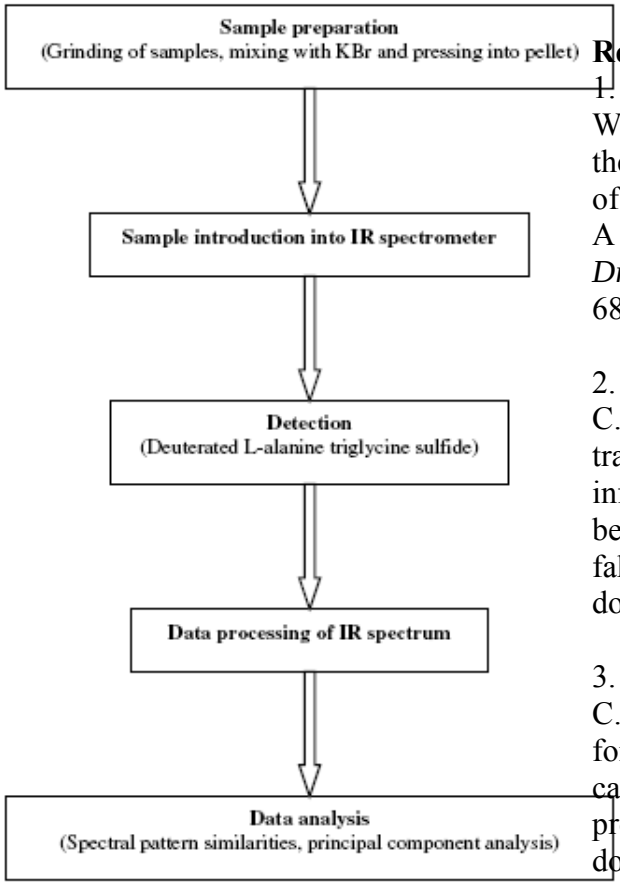

Fig. 1. Quality surveillance protocol for ginseng based on IR spectral wavelengths.

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Microwave-induced melt agglomeration-comparison with conventional melt agglomeration

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Introduction

In spite of the rising popularity of microwave technology[1], its application to pharmaceutical unit processes, in particular melt agglomeration, has yet been explored. The objective of this study was to demonstrate the feasibility of microwave-induced melt agglomeration. Comparisons were made between melt agglomeration using conventional and microwave heating. Particular attention was paid on the correlation between in-process parameters derived and sizes of resultant melt agglomerates.

Experimental

The materials used were lactose 200M (Pharmatose 200M, DMV, the Netherlands), anhydrous dicalcium phosphate (DCP, Rhodia, USA) and polyethylene glycol (PEG 3350, Clariant, Germany). Lactose was pre-sieved prior to use. The particle sizes (D50), moisture contents and bulk densities of the raw materials are shown in Table 1.

Microwave-induced melt agglomeration of an admixture comprising equivalent proportions by weight of lactose 200M and DCP was carried out in a 10L high shear single pot processor (UltimaPro 10, Collette, Belgium) using 20 %w/w (with respect to total weight of admixture) of PEG 3350. The total weight of the admixture was kept at 1000 g. Powders were first homogeneously mixed in the mixer bowl that was preheated to 50 ºC. Upon equilibration to the bowl temperature, they were exposed to microwaves (900 W) and subjected to high shear massing at an impeller speed of 630 rpm until PEG melted as indicated by a rise in mixer power consumption. High shear massing was continued for post-melt massing times (PMMT) of 10, 14, 16 and 18 min. This was followed by a 4 min low shear massing phase at an impeller speed of 500 rpm and without microwave input. Outputs for mixer power consumption and product temperature were continuously recorded during the process.

Conventional melt granulation was carried out under the influence of thermal energy supplied by the mixer bowl. The procedure was identical to that aforementioned except that energy was derived from the mixer bowl jacket heated to 60 ºC. A minimum of 3 replicate runs were carried out at each PMMT. The melt granulates prepared using conventional and microwave heating would be abbreviated as 'CMG' and 'MMG' respectively.

Resultant granulates were sieved through a 2.80 mm sieve to remove the lumps. These lumps and the undersize fraction of granulates were cooled to ambient temperature by spreading them in thin layers on trays. Granulates smaller than 2.80 mm were sub-divided by a spinning riffler (PT, Retsch, Germany) and their size distribution determined by sieving. The mass median diameter (D50), % lumps and yield of each batch of granulates produced were computed.

The peak mixer power consumption (MP_{peak}) as well as the mixer power consumption (MP_{HS}, MP_{LS}) and product temperatures (PT_{HS}, PT_{LS}) at the end of the high shear and low shear massing phases were extracted from the mixer power consumption and product temperature profiles. In addition, the average post-melt specific power consumption, P_{av}, was computed. P_{av} represents the work done per kg of powder during the post-melt phase of granulation. The influence of these parameters on the D50 and % lumps of granulates produced using the two methods were assessed.

Table 1. Physical properties of materials used.

Material	D50[a] (µm)	Moisture content[b] (%)	Bulk density[c](g/ml)
Lactose 200M	36.83	0.236	0.43
DCP	15.84	0.225	0.70
PEG 3350	255.18	0.596	0.59

[a]LS230 with dry powder feeder, Coulter, USA
[b]Thermogravimetric analysis, DTG 60H, Shimadzu, Japan
[c]Stav 2003, JEL, Germany

Results and discussion

The influence of PMMT on the D50 and % lumps for both MMG and CMG are shown in Figs.1a-b. For both granulates, increased PMMT did not result in proportionate increases in D50. At identical PMMT, the D50 and % lumps of CMG were higher than that of MMG. The excessively high proportion

of lumps for CMG were suggestive of uncontrolled or ball growth.

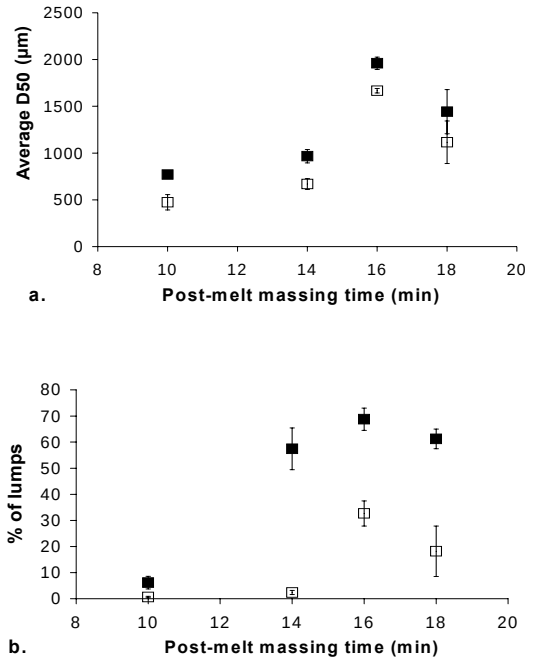

Fig. 1a-b. Effect of PMMT on D50 and % lumps of (□) MMG and (■) CMG.

Amongst the various parameters used, D50 of MMG was found to be significantly correlated to the peak mixer power consumption (MP_{peak}) as well as the mixer power consumed at the end of the high (MP_{HS}) and low shear (MP_{LS}) massing phases. P_{av} also increased proportionately with D50 (Table 2). These results suggested that agglomerate growth under the influence of microwaves occurred in a more controlled and predictable fashion. Furthermore, D50, % lumps and yield of MMG were found to be inter-related. Increased D50 was accompanied by increased % lumps ($r=0.994$, $p<0.01$). The latter was in turn inversely related to % yield ($r=-1.000$, $p<0.01$).

Table 2. Correlation coefficients of D50 and % lumps with the in-process parameters

In-process parameters	$D50_{MMG}$	$D50_{CMG}$	% $Lumps_{CMG}$
MP_{peak}	0.989*	0.624	0.978*
MP_{HS}	0.992**	0.214	0.748
MP_{LS}	0.998**	-0.223	0.265
PT_{HS}	0.626	0.820	0.989*
PT_{LS}	0.884	0.769	0.998**
P_{av}	0.968*	0.838	0.964*

*Correlation significant at 0.05 level
**Correlation significant at 0.01 level

On the contrary, no relationship was found between D50 of CMG and the various derived parameters. (Table 2). This was likely to be due to the uncontrolled growth of CMG under the prevailing experimental conditions resulting in overgrowth and a significant proportion of lumps at the end of the process. Further correlation studies carried out revealed a strong association between the % lumps of CMG and peak mixer power consumption (MP_{peak}) as well as the product temperatures at the end of the high (PT_{HS}) and low shear (PT_{LS}) massing phases. % lumps was also found to be related to P_{av} (Table 2).

Conclusions

The feasibility of microwave-induced melt agglomeration of an admixture of lactose 200M and DCP using 20 %w/w PEG 3350 in a high shear single pot processor had been demonstrated. Under identical processing conditions, agglomerate growth under the influence of microwaves was generally more controlled, resulting in smaller granulates and reduced % lumps compared to those produced under conventional heating. Furthermore, growth of MMG could be easily predicted from the mixer power consumption profiles during granulation.

It was likely that the range of PMMTs employed were responsible for the uncontrolled growth of CMG. Further optimization studies for conventional melt granulation would be carried out.

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INFLUENCE OF USERS AND STORAGE CONDITIONS ON CONTAMINATION AND DISINFECTANT EFFICACY OF CONTACT LENS SOLUTIONS

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Introduction

Contact lenses provide an alternative to the conventional spectacles and 9% percentage of the myopic population in Singapore uses contact lenses.[1] There are many causes leading to contact lens related eye infection and complications arising from lens care are usually multi-factorial. The failure of the disinfectant solution to properly eliminate microorganisms could be a result of the habits of contact lens wearers and their knowledge on lenses and lens care products.

Experimental

26 students from National University of Singapore (Arts, Engineering, Pharmacy and Science) were supplied with Complete® Moisture Plus Multi-Purpose Solution™ for a 10-week study. Samples were collected and the extent of contamination was evaluated using BP test for viable count[2] at weeks 4, 6 and 10 and swabbing of bottle surfaces at the end of the study. Disinfectant efficacy was evaluated by criteria stated in the US-FDA Guidelines.[3] Contact lens solutions were also stored at various locations in the house to determine the effect of storage conditions on disinfectant efficacy. In addition, a survey was conducted to examine lens wearers' knowledge and practices in lens care and compliance to standard recommendations of lens care.

Results and discussion

Contamination levels were comparably lower for samples from the Arts and Pharmacy faculties than that of Science and Engineering faculties. Most common microorganisms identified were gram-negative rods (52.2%), gram-positive cocci (21.1%) and molds. Participants generally demonstrated a high level of hygiene and compliance to recommended regimens of lens care. No statistical significance was observed for differences in disinfectant efficacy of contact lens solutions over time among various faculties ($p > 0.05$) although significant difference was noted for storage in the bathroom ($p < 0.05$). Most contact lens wearers surveyed responded their contact lens solutions were stored in the bathroom (36.7%).

Conclusion

Contact lens practitioners should reiterate to contact lens wearers the importance of compliance to recommended disinfecting steps in lens care and storage of contact lens solutions.

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DISINFECTANT EFFICACY AND USAGE OF CONTACT LENS SOLUTIONS IN SINGAPORE

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Introduction

The prevalence of myopia globally has seen a surge of people needing refractive correction. Currently there is an estimated 125 million contact lens wearers worldwide, with 0.32% of them in Singapore[2].This trend is likely to continue increasing. Contact lens care requires a whole host of considerations from the quality of contact lens and contact lens care products to the usage by the wearer. A lapse in any of these aspects may lead to potential eye complications. The recent *Fusarium* keratitis outbreak was attributed to the formulation of a widely-used multi-purpose solution affecting its disinfecting ability, coupled with suboptimal user's hygiene in handling contact lens products.

Experimental

A study was carried out on the disinfectant efficacy of contact lens solutions and users' knowledge in contact lens care. Eleven contact lens solutions were challenged with *Pseudomonas aeruginosa* and *Staphylococcus aureus.* Disinfectant efficacies of the solutions were evaluated using a procedure adopted from the FDA Stand-alone Test. A survey was conducted on lens wearers aged between 13-25 years to determine user's lens care knowledge, lens care practices and compliance with practitioners' advice. Trends in practitioners' lens care recommendations and adequacy of such advice were also evaluated using a separate survey for practitioners.

Results and discussion

All contact lens solutions, except ReNu® MoistureLoc™, met the primary criteria of the test against the challenge organisms. There was no difference in disinfectant efficacies between the various formulations against *Ps. aeruginosa*. The disinfectant efficacies against *S. aureus* were lower compared to *Ps. aeruginosa.* The various solutions, including those containing similar disinfectant concentrations, resulted in different log reduction values against *S. aureus*. The surveys showed that users were generally well-equipped with lens care knowledge. Areas of non-compliance included replacement frequency of contact lens solutions and cases which were often neglected by practitioners during dispensing of lens care advice.

Conclusion

Overall, the contact lens solutions in the local market were found to be efficacious, although the type of disinfectant and additives in the formulation may affect its disinfectant efficacy. Practitioners should better equip users with lens care knowledge, as well as encourage compliance with recommended care regimens.

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Antioxidant activity of standardized extract from *Gastrodia elata*

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Introduction

Gastrodia elata (GE) is a popular used traditional medicine in China, and it is well known due to its anti-migraine and anticonvulsant activity. Quite a few publications have reported that ether fraction of methanolic extract (EFME) of GE showed neuroprotective activity. Besides, some small molecule phenolic compounds were isolated and identified from this fraction. Since phenolic compounds are widely recognized as free radical scavengers, especially flavonoids, it is interesting to understand the antioxidant activity of these small molecule phenolic compounds from GE, hoping that antioxidant activity of EFME could explain its neuroprotective action. To confirm that EFME is composed of phenolic compounds, the total phenolic content was determined using Folin-Ciocalteu method, and this fraction was standardized against two commercially available standards, 4-hydroxybenzyl alcohol (4HA) and 4-hydroxybenzaldehyde (4HD) by RP-HPLC. The antioxidant activity of EFME was also evaluated by DPPH free radical assay.

Experimental

Preparation of EFME from GE

Wild and cultivated type of GE, bought from Sichuan province of China, and two commercial products contained exclusively GE, purchased in Singapore, were milled and extracted by methanol in reflux. The methanolic extracts were dried under vacuum then suspended in water to be extracted by diethyl ether, and ether fraction were dried under vacuum and redissolved in an appropriate volume of methanol for assay use.

Determination of total phenolic content in EFME

Calibration curve was prepared from different concentrations of gallic acid solution. Water, Folin-Ciocalteu reagent and saturated sodium carbonate solution were sequentially added into 10ml volumetric flask, diluted to 10ml with water. Mixture was allowed to react in the dark for 1hr. The absorbance of final solution was measured at 725nm using UV-visible spectrophotometer. Results were expressed as gram of gallic acid equivalent (GAE) per 100g of dry weight (DW) of the extracts.

Standardization of EFME against 4HA and 4HD

The quantity of 4HA and 4HD were determined by RP-HPLC, and samples were eluted by gradient procedure with water and acetonitrile as mobile phase, and flow rate was 0.5ml/min. 4HA was detected under 230nm, whereas 4HD was detected under 270nm.

DPPH free radical assay

Samples were added to DPPH· solution, and the decrease in the absorbance at 515nm was determined using a UV-Visible Spectrophotometer after two hours when the reaction reached the steady state in the dark. The remaining DPPH· concentration in the reaction medium was calculated from the DPPH· calibration curve. Results were expressed as standard equivalents using quercetin and Trolox on the basis of the EC_{50} value ($EC_{50\ quercetin/Trolox}$/ $EC_{50sample}$).

Results and Discussions

The total phenolic content of EFME from four samples was 5-7g gallic acid per 100g using Folin-Ciocalteu method. Compared

with the total phenolic content of most medicinal herbs (0.22-50.3%), it showed that EFME contained a certain amount of phenolic compounds but the quantity was not impressive. By means of RP-HPLC, it was found that there was 4.16% 4HA and 1.2% 4HD in wild type of GE, in contrast, only 0.69% 4HD but no 4HA were found in cultivated type. Interestingly, it was found out that GE product branded as Meihua contained 1.35% 4HA and 1.47% 4HD, and there was 1.65% 4HA and 1.06% 4HD in Nature's Green brand. It is possible that the source of these two brands is miscellaneous from cultivated and wild type of GE.

By using DPPH test, we have found that there was weak antioxidant activity of EFME from GE. After statistically analyzing four samples, no significant difference in the total phenolic content was observed, while significant difference was indicated for their antioxidant activity. This incompliance may indicate that there are other compounds which possess antioxidant activity as well. In addition, the antioxidant activity of 4HA and 4HD were also evaluated by DPPH test. Turning to the above result of standardization, it is found that quantity of 4HA and 4HD contained in EFME is too low to explain all antioxidant activity owned by this fraction. Thus, phenolic compounds in EFME are not fully responsible for its antioxidant activity. Other components in this fraction also take a part.

Conclusion:

EFME of GE contained phenolic compounds which could play a part in its antioxidant activity that may further contribute to the neuroprotective effects.

CHALCONES: POTENTIAL MODULATORS OF BCRP-MEDIATED MULTIDRUG RESISTANCE

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Introduction

Multidrug resistance (MDR) poses a significant challenge to successful chemotherapy and is often mediated by the overexpression of ATP-binding cassette (ABC) transporters, which efflux anticancer drugs via an energy-dependent mechanism, thereby limiting intracellular drug concentration. One recently identified member of the ABC transporter superfamily is the breast cancer resistance protein (BCRP). Current measure to overcome MDR is through the use of transport inhibitors that increase the intracellular accumulation of anticancer drugs, restoring chemosensitivity. Some examples of BCRP inhibitors include fumitremorgin C, GF120918, estrogens such as estrone and 17β-estradiol, Tryprostatin A, and flavonoids. In the present study, we investigated a class of compounds closely related to the flavonoids, the chalcones, for their potential to act as BCRP inhibitors. This may be attributed to favourable hydrophobicity, planarity, and steric/shape configuration, all of which have been shown to promote drug-BCRP interaction. These findings could be useful for predicting BCRP inhibitory activity of other untested chalcones and for guiding the synthesis of potent BCRP inhibitors for potential clinical application.

Experimental

The overexpression of BCRP in the cells was tested with Western Blot analysis. Following that, we investigated the ability of chalcones 1-13 to mediate BCRP inhibition through accumulation (flow cytometric analysis) and cytotoxicity (MTT assay) studies, using mitoxantrone, a well-known BCRP substrate. Based on the preliminary screening of the 13 chalcone compounds, we further synthesized 4 chalcone derivatives (chalcones 14-17) to fully elucidate the structure-activity relationship (SAR) between chalcones and BCRP inhibition.

Results and Discussion

We found that the presence of certain structural motifs resulted in greater accumulation of drug in the cells and thus greater cell cytotoxicity. These motifs emphasize the importance of a hydrophobic, conjugated, planar ring, as pointed out in earlier studies (1,2). Based on the SAR analysis, we concluded that 2',4'-OCH_3 of Ring A, 2'-OH of Ring A, and Cl at position 4 of Ring B are important structural constituents for potent chalcone-BCRP interaction.

Conclusion

Our study has shown that chalcones, at relatively low concentrations, were effective in inhibiting BCRP-mediated efflux, resulting in an increase in the cellular accumulation of BCRP substrates, thereby reversing MDR. Based on the SAR studies, it may be concluded that certain structural motifs are crucial for effective chalcones-BCRP interaction. A more thorough SAR analysis would be necessary for designing more potent chalcones as BCRP inhibitors for potential clinical application.

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Nano liposomal encapsulation and in vitro activity study of a new DHFR inhibitor

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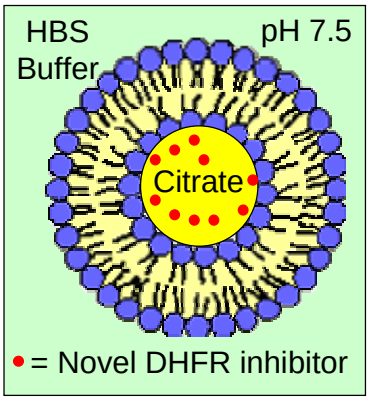
Introduction

In patients with advanced breast cancer, antimetabolites such as methotrexate and 5-fluorouracil are commonly employed as monotherapy or in combination with other agents for cytotoxic chemotherapy. However, for methotrexate which is a dihydrofolate reductase (DHFR) inhibitor, there is a lack of selectivity which results in systemic toxicity. In response to this problem, researchers have recently created a new DHFR inhibitor, M-V-05, which is more tumor selective. It is postulated that encapsulating the novel drug inhibitor in a liposomal formulation will further enhance tumor selectivity as the liposome delivery system will confer the ability to target the chemotherapy to cancer cells. This is theoretically possible by the combination of the intrinsic selectivity of the novel inhibitor on the cancer cells with the enhanced permeability and retention effect of liposomal drugs at the tumor site.

Experimental

Hence, in this project, the main focus was the development of the liposomal formulation of the novel drug. The pH gradient loading technique was used to load the drug into the liposomes, and formulation stability was assessed based on percentage retention of drug, liposome size and polydispersity. Anti-proliferative activity of liposomal M-V-05 on breast cancer cell lines was characterized and compared to that of free drug.



Results and discussion

The results indicate that the novel DHFR inhibitor could be encapsulated effectively with a loading efficiency of approximately 90%, via the pH gradient loading mechanism into liposomes. The liposomal formulation displayed stability under physiological, ambient and refrigerator storage temperatures and exhibited anti-proliferative effects towards a breast cancer cell line.

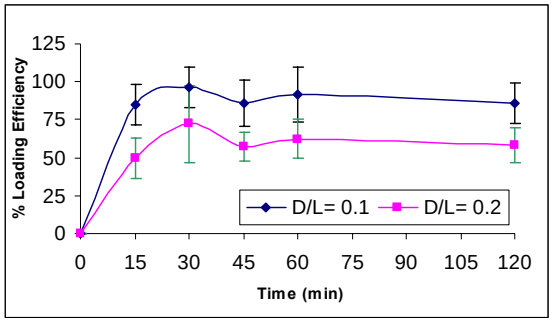


Figure 2. Percentage loading efficiency profiles of M-V-05 into liposomes over time. Two D/L weight ratios of the initial reaction mixture were investigated (0.1:1, ♦; 0.2:1, ■). Percentage loading efficiency at specific time points was calculated as follows: percentage loading efficiency = (Final D/L) / (Initial D/L) x 100%.

Conclusion

These results demonstrated the therapeutic potential of liposomal M-V-05 for further evaluation in breast cancer.

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Biofunctionalized Thermosensitive Membranes for the Self-renewal of Adult Stem Cells

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Introduction:

Stem cell therapy using adult human bone marrow-derived mesenchymal stem cells (hMSC) presents a novel approach for tissue repair and regeneration. Its success depends on the availability of efficient techniques for *in vitro* expansion and recovery of hMSC, and the development of suitable scaffolds for the transplantation of hMSC into damaged tissues. Previously, novel nanostructured, thermosensitive membranes, containing Poly(N-isopropylacrylamide), were developed for hMSC expansion and harvest. These membranes have been shown to facilitate hMSC proliferation and differentiation, and allow spontaneous detachment of cells in response to cold treatment of 15-20°C, without any compromise in cell integrity. They could also be employed as 2D scaffolds for hMSC delivery and transplantation, especially in the area of wound healing. Unfortunately, the potential of the membranes was limited by the poor attachment and low proliferation rate of hMSC on the membrane. In the present study, the thermosensitive membranes are biofunctionalized with either type I collagen or arginine-glycine-aspartate (RGD) peptides to promote hMSC attachment and proliferation for future applications in stem cell therapy.

Experimental:

Surfaces of presynthesized membranes were modified either by the physical adsorption of a thin collagen film or by the grafting of arginine-glycine-aspartate (RGD) peptides using an aqueous phase reaction. The functionalized thermosensitive membranes were characterized and assessed for their ability to improve hMSC attachment and proliferation.

Further, to verify that the thermosensitive properties of the membranes remained unaltered, the ability of hMSCs to spontaneously detach from the modified membranes when ambient temperatures was reduced to 15°C was tested.

Results and discussion

hMSC attachment and proliferation on collagen- and RGD-modified membrane surfaces were found to be increased, with the latter stimulating comparatively better attachment. Collagen modification, however, induced a more pronounced improvement in hMSC growth compared to RGD-modified surfaces. Cultured hMSCs on both types of surfaces maintained their characteristic morphologies, demonstrating that their multipotency was preserved and that interactions with the proteins/peptides did not stimulate them to differentiate. Modification with RGD peptides did not affect the high efficiency of hMSC detachment, which was in contrast to the slightly compromised hMSC detachment from collagen–modified membranes.

Conclusion

Novel thermosensitive membranes biofunctionalized with type I collagen or RGD peptides have great potential for use in the scale-up expansion and recovery of hMSCs and as scaffolds for the delivery of hMSC for regenerative stem cell therapies.

THERMAL ANALYSIS AND PERMEATION STUDY ON THE HUMAN SKIN LIPIDS AND FABRICATION OF SKIN BARRIER

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Introduction

The human stratum corneum (SC), the outermost layer of the skin, acts as the main barrier to the external environment, even though it's a very thin layer of 10-20 μm, and consists mainly of corneocytes embedded in a matrix of multi-cellular lipid bi-layers (consisting largely of cholesterol, ceramides, and free fatty acids).

Heat and moisture are known factors that influence the structure and permeability of the SC. However, much is still unknown about the mechanism of how these factors work.

The objective of the Thermal Analysis Microcalorimetry (TAM) study was to obtain the thermoprofiles of the SC lipids under varying RH and skin temperatures and relating them to both the observed and reported effects of humidity and temperature on the SC. Greater insights on the structure of the SC and how the human skin would change in response to changes in the environment, for example during thermoregulation.

The objective of the permeation study is to compare the permeation of HP across the membranes of different lipid compositions, in order to elucidate the roles of some of these lipids by fabrication of lipid membranes to simulate the SC for in vitro percutaneous penetration studies.

The interaction of water vapour and the lipids was investigated over a range of skin temperatures (32 - 42^{o}C) using Isothermal Microcalorimetry (TAM) to attain thermoprofiles of different SC lipids at various relative humidities (RH).

Fabricated membranes of several combinations of SC lipids were constructed in an attempt to identify the critical components for fabrication of a substitute SC membrane for *in vitro* drug permeation modelling, using Haloperidol (a model drug) with flow-through diffusion cells.

Results and Discussion

Our results showed that water vapour did not significantly interact with either the ceramides or the free fatty acids at any RH. However, cholesterol forms hydrates with water at 100% RH, and the process is temperature-dependent with a peak at 37^{o}C. Thus we identified a source of interaction that could explain the variable low peak in DSC studies on SC that is highly debated. We also suggest that the increased hydration results in increased formation of cholesterol monohydrate. Thus form crystalline lipid rafts in the SC lipid lamella disrupting the packing of the lipid lamella increasing the disorder of the SC, allowing for increased permeation of drugs through hydrated skin.

The *in vitro* drug permeation study showed that the lipid composition was important in determining the barrier

properties of the membrane in the drug permeation profiles. the cholesterol embedded membranes showed the lowest flux, and the cholesterol-free-fatty-acid embedded membranes showed similar drug permeation profile. The ceramide-cholesterol-free-fatty-acid membrane showed a higher flux than both the above membranes. The results showed that ceramides and cholesterol could play a significant role in the barrier properties of SC. However further investigations is needed to improve on the membrane fabrication for a SC substitute.

Upon a 2-by-2 analysis approach on the drug permeation profiles of cholesterol membranes at 32^{o}C and 37^{o}C, temperature showed a significant effect on the drug permeation profile with an increase in flux. Cholesterol showed a significant effect on permeation profile by decreasing flux, confirming cholesterol could be important in the SC barrier function for the permeation of drugs. Cholesterol showed significant antagonistic interaction with temperature, which tallies with our TAM study.

Conclusion

From our TAM studies at the range of human vapour did not interact significantly with either the ceramides or the free fatty acids the range of RH. However, cholesterol formed complexes with water at 75 to 100% RH, with a peak at 37^{o}C. This helped to explain the low peak of the SC observed in DSC studies, the lipid structure of the SC and how cholesterol could play a role in the thermoregulation of skin temperature.

The *in vitro* permeation studies have showed that cholesterol and ceramides were important components for fabrication of membranes to mimic the drug permeation profile through the SC. Temperature and cholesterol played significant roles in the permeation of haloperidol and with a significant interaction between these factors. However the membranes in our study have to be improved upon before they can become SC substitutes.

In Vitro Assessment of Synergistic and Antagonistic Combinations of Cytotoxic Agents in Ovarian Cancer Cell Lines

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Introduction Ovarian cancer is one of the most fatal gynecological cancers and is estimated to cause 14,461 US and 124,860 worldwide deaths in 2002. The current first line chemotherapy regimen for patients with ovarian cancer includes a combination of paclitaxel plus a platinum agent. Unfortunately, substantial risk of recurrence and emergence of drug-resistant disease are problems associated with platinum-based therapy. Therefore, there is an urgent need to develop novel treatment strategies to curb the progress of this fatal disease. It is hypothesized that combinations of cisplatin with histone deacetylase inhibitors (HDACIs) or Arsenic Trioxide with HDACIs will have synergistic effects in the treatment of ovarian cancer.

Experimental SKOV-3 and OVCAR-3 ovarian cancer cell lines were subjected to 4 treatment combinations of cytotoxic agents namely: Arsenic Trioxide/MS-275, Arsenic Trioxide/SAHA, cisplatin/MS-275 and cisplatin/SAHA. The median-drug effect analysis method was used for evaluation of potential synergism between the 2 different agents in each combination. Apoptosis was assessed using flow cytometry.

Results and discussion Cisplatin with either MS-275 or SAHA demonstrated strong synergistic cytotoxicity on both ovarian cancer cell lines. The combinations of Arsenic Trioxide with either MS-275 or SAHA were antagonistic. It is thus likely that modulation of the acetylation status generated by the concurrent treatment with HDACIs increased the efficiency of the cytotoxic agents targeting DNA. Flow cytometry analysis showed that when the cells were treated by the cytotoxic agents alone, there were slight increases in the percentage of the apoptotic cells. However, when cisplatin was used in combination with MS-275 or SAHA, a statistical significant increase in the percentage of apoptotic cells was seen. It is likely that the cytotoxic enhancement observed for concurrent treatment is associated with activation of apoptotic pathways.

Conclusion Due to the heterogeneity of cancer cells and thus the frequent development of resistance, seldom are any chemotherapeutic agents used singly. The study had identified the combinations of cisplatin/MS-275 and cisplatin/SAHA to show strong synergistic cytotoxicity on SKOV-3 and OVCAR-3 ovarian cancer cell lines. This suggests that adding HDACIs to ovarian cancer chemotherapy could increase the efficacy of treatment.

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Impact of Cultivation/Maintenance Techniques and Conditions on Microorganisms

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INTRODUCTION

Each microbial species grows best in a particular set of environmental conditions and exhibits a certain extent of adaptability and tolerance to the environmental conditions.[1] In the cultivation and maintenance of microorganisms, temperature, osmotic pressure, pH, and composition of culture media can potentially affect their growth and conditions; hence affecting standardization of test inoculum.[2,3] *Ps. aeruginosa* is commonly used in microbiological tests, but there are limited studies on the factors affecting its growth.

EXPERIMENTAL

Effect of Temperature and Culture Medium on Microbial Growth

Fourth subculture of *Ps. aeruginosa* was incubated at different combinations of temperature and culture media for 24 hours. Total viable count was determined by surface plating method. The extent of microbial growth was expressed as the mean log growth index, which was calculated as follows:

$$\text{Log Growth Index} = \log\left(\frac{\text{Viable Count after 24 - hour Incubation}}{\text{Viable Count before Incubation}}\right)$$

Effect of Different Suspending Agents on Viability of Microbial Cells

Fourth subculture of *Ps. aeruginosa* were harvested using different suspending agents (buffered sodium chloride-peptone water,[4] 0.9% sodium chloride solution, and sterile water) and stored at 4 ^{0}C.

Viable count (VC) was determined at different time intervals. The viability of the microbial cells was expressed as the mean percentage change in viable count, which was calculated as follows:

$$\text{Percentage Change in Viable Count after X Day(s)} = \left(\frac{\text{VC at Day X - VC at Day 0}}{\text{VC at Day 0}}\right) \text{x } 100\%$$

Effect of Number of Subcultures on Morphology and Microbial Growth

Fourth to tenth subcultures of *Ps. aeruginosa* were studied. The extent of microbial growth was expressed as mean log growth index. Samples (n=250) from each subculture were stained using Gram's staining method,[2] and examined microscopically with the image analyser. Cell morphology was determined by mean area for size and mean aspect ratio for roundness. The aspect ratio is expressed as the ratio of the longest axis of cell to the shortest axis of cell.[5]

Effect of Storage/Maintenance Conditions on Viability of Microbial Cells

Test combinations were inoculated with fourth and tenth subcultures and incubated using four different storage media and two different cultivation vessels at 37 ^{0}C before being stored at 4 ^{0}C. Total viable count was determined at different time intervals. The viability of the microbial cells was expressed as the mean percentage change in total viable count.

RESULTS AND DISCUSSION

Increases in cultivation temperature from 25 ^{0}C to 37 ^{0}C resulted in increased rates of enzymatic reactions and extents of bacterial growth.[6] The liquid media used exhibited significantly greater extent of microbial growth than the solid media used, due to higher availability of dissolved nutrients in liquid media that are more easily assimilated by the bacteria. Tryptone soya media exhibited significantly greater extent of microbial growth than nutrient media, due to the additional vitamin complexes, and nitrogen sources essential for growth and cellular replication.[3]

Continuous increases in viability noted in bacterial cells suspended in buffered sodium chloride-peptone water (PW) could be due to the presence of water-soluble nitrogen in PW. Declines in viability of bacterial cells suspended in NaCl solution and sterile water after the initial increases could be due to the absence of growth-supporting nutrients, as well as the pH and osmolarity of the suspending agents.

Significant correlation was observed between the extent of microbial growth and the size of the microbial cells over the changes in number of subcultures, but not between the extent of microbial growth and the shape of the bacterial cells.

Tryptone soya broth exhibited the lowest reduction in viability after 7 days and 14 days, and was the optimum storage media. As the bacterial surface structures varied with the environmental temperature and the bacterial membrane lipid structure was modified continuously with the change in temperature to maintain lipid function,[3] *Ps. aeruginosa* could have developed surface structures that did not enable the increased uptake of additional nutrients present in tryptone soya broth, or altered membrane structures that impaired or slowed the nutrient uptake. The growth factor requirement for bacteria was reported to change with temperature.[3]

CONCLUSION

In this study, the growth of *Ps. aeruginosa* was found to be significantly affected by both cultivation temperature and type of culture medium, as well as by the number of subcultures and type of suspending agent. The viability of *Ps. aeruginosa* on storage was significantly affected by the type of culture medium and suspending agent, as well as the duration of storage/maintenance. There was no significant effect of number of subcultures and type of cultivation vessel on the cell viability.

By understanding the impact of various cultivation-related and storage/maintenance-related factors on the extent of microbial growth and viability of *Ps. aeruginosa*, recommendations and guidelines for the cultivation and storage/maintenance of the bacteria can be established. With proper standardization and control of microbiological techniques in cultivation and storage/maintenance of *Ps. aeruginosa*, standardisation of the test inoculum can be achieved to obtain more consistent results in microbiological tests.

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Better drug uniformity of Bottom spray compared to Top spray fluidized bed granulation granules

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Introduction

Fluidized bed granulation (FBG) can be divided into top spray granulation (TG) and bottom spray granulation based on the orientation of the spray nozzle. An example of the latter is precision granulation (PG) as shown in figure 1 (Walter and Schurter).

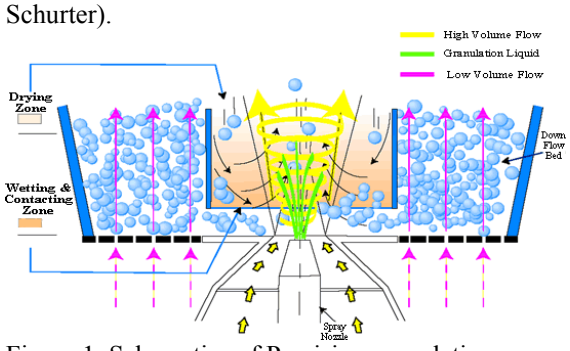

Figure 1. Schematics of Precision granulation.

Properties of starting materials such as size and flow, and the mode of drug distribution during granulation may affect granule quality which may in turn influence drug content uniformity in tablets. As such, it was hypothesized that drug distribution and drug uniformity in granules could be influenced by the techniques of FBG and the physical characteristics of the starting material used for granulation. In this study, attempts were made to prepare a series of lactose powder blends with varying particle sizes and flow properties by doping lactose 200M (a lactose grade commonly used for FBG) with lactose 450M and 150M. These blends were used to model a starting material of the same type but with different physical properties to investigate the influence of the mode of deposition of a micronized drug via the binder fluid using PG and TG processes.

Experimental

Lactose monohydrate (Pharmatose 150M, 200M and 450M, DMV, The Netherlands) was used as the feed material for the granulation processes. The aqueous liquid binder consisted of two grades of polyvinylpyrrolidone (PVP) (Povidone K25 and Povidone K90, ISP, USA) and hydrochlorothiazide (HCT).

Lactose 200M was doped with varying percentages of lactose 450M and 150M to give a range of lactose powder blends, coded as formulation 1-9, in table 1.

Table 1. Composite of the different lactose formulas.

Formula code	Lactose 200M (%)	Lactose 450M (%)	Lactose 150M (%)
1	0	100	0
2	50	50	0
3	60	40	0
4	70	30	0
5	80	20	0
6	90	10	0
7	100	0	0
8	90	0	10
9	0	0	100

The different grades of lactose powder (were weighed out and mixed in a double-cone mixer (AR 400E, Erweka, Germany). The particle mean size and size distribution (span) of the different lactose powder blends were determined using a laser diffraction sizer (LS 230, Coulter Corporation, USA). Powder flow on the lactose blends was carried out using a powder tester (PT-N, Hosokawa Micron, Japan), from which the angle of repose was determined.

PG and TG modules were fitted onto the air handling system (MP-1 Multi-processor, GEA Aeromatic-Fielder, UK), under standardized conditions. Granules prepared by both processes were analyzed for their size, shape and drug content. The mass median diameter (MMD), span, and drug uniformity of the granules were then determined.

Results and discussion

As shown in table 2, when the percentage of lactose 450M decreases (formulas 1 to 7) and lactose 150M increases (formulas 7 to 9) in the lactose blends, there was an increase in mean size and an improvement in flow. Formulas 1-8 were then chosen for testing in both processes to evaluate if they could be granulated. The last formula was not included as its improvement in flow was statistically insignificant ($p>0.05$). It was observed

Table 2. Mean size, span and angle of repose of the different formulas.

Formula	Mean size (μm)	Span	Angle of repose (°)
1	25.65(1.71)	1.87(0.12)	60.2(0.4)
2	30.25(1.66)	1.56(0.11)	56.9(0.5)
3	32.77(3.73)	1.70(0.16)	56.2(0.4)
4	33.51(0.01)	1.66(0.05)	55.1(0.2)
5	34.19(0.34)	1.33(0.01)	54.2(0.5)
6	37.65(0.94)	1.64(0.04)	52.4(0.4)
7	38.99(0.01)	1.71(0.07)	49.5(1.2)
8	42.93(0.32)	1.77(0.11)	48.1(0.2)
9	73.40(3.56)	2.22(0.29)	47.9(0.2)

that only formulas 4 to 8 were suitable for granulation in both processes and thus were chosen to be studied thereafter. As formulas 4 and 8 were the starting materials with the smallest and largest particle sizes, they were selected for drug content analysis and shape analysis among the five.

Generally, TG produced larger granules than PG (table 3). This is because TG has a larger spray zone which forms a larger nucleation zone. The fine powders were more effectively wetted and nucleated in the larger nucleation zone, leading to the formation of larger granules. Thereafter, the nuclei coalesced and consolidated to form agglomerates when they collided with each other. However, in PG, the powders had to follow a queue system into the spray zone to be wetted and they were usually almost dried when they left the partition column. This led to a drier granulation process and comparatively, a slower granule growth.

Table 3. Size characteristics of PG and TG granules.

Process	Formula	MMD (μm)	Span
PG	4	420(111)	1.36(0.07)
	5	477(179)	1.46(0.31)
	6	375(46)	1.30(0.03)
	7	422(16)	1.29(0.13)
	8	327(10)	1.23(0.03)
TG	4	548(46)	1.35(0.30)
	5	632(98)	1.42(0.19)
	6	477(43)	1.57(0.05)
	7	525(55)	1.33(0.18)
	8	440(101)	1.02(0.08)

The mass drug content of TG granules was found to be generally higher than that of PG granules (table 4). This could be due the lower process yield in TG compared to PG which was observed during the study. The results also suggested that formula 4 had poorer inter-batch (table 4) and intra-batch (figure 1) drug uniformity than formula 8 for both PG and TG. This could be due to the smaller mean particle size in formula 4 which resulted in an increased cohesiveness between the lactose particles and less ease in fluidization. When the fluidization was poor, the mixing of binder liquid and powder was less uniform. Thus, some granules were loaded with more drugs than others, giving rise to the lower drug uniformity.

Table 4. Drug content of PG and TG granules prepared with formulas 4 and 8.

Process	Formula	Mass drug content (%w/w)	Inter-batch drug uniformity (%)
PG	4	1.95 ± 0.10	5.31
	8	1.86 ± 0.07	4.01
TG	4	2.23 ± 0.43	19.39
	8	2.24 ± 0.29	12.93

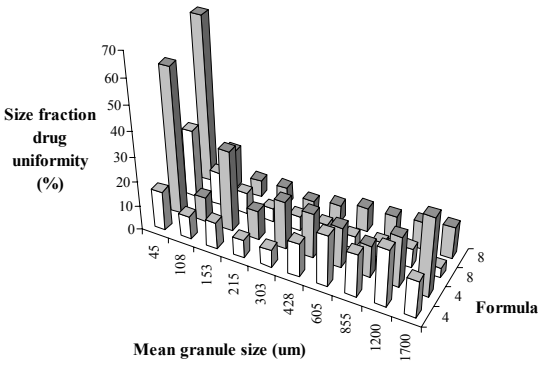

Figure 1. Drug uniformity within different size fractions of PG (□) and TG (■) granules.

The inter-batch and intra-batch drug content of PG granules were observed to be more uniform than that of TG granules and this indicated a more reproducible process in PG.

Conclusion

The differences in the size and flow properties of starting material did not appear to influence granule growth in PG and TG. Granules of lower intra-batch and inter-batch drug uniformity resulted from starting material with a smaller mean size and poorer flow. The characteristics of starting material and the spray orientation of the binder fluid are important considerations when a micronized drug is to be delivered via the binder fluid during FBG. PG was observed to be a better process to employ compared to TG.

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Iron oxide nanoparticles for biomedical applications: Development of fluorescein-loaded and oleic acid-pluronic-coated iron (III) oxide nanoparticles as potential multimodal nanoparticles

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Introduction

Fluorescein-labeled iron (III) oxide nanoparticles were developed with potential application as a multimodal system for both fluorescence optical and magnetic resonance imaging (MRI). Multimodal nanoparticles could also offset the limitations of both MRI and fluorescence optical imaging (1). Fluorescence optical imaging has high sensitivity, but cannot provide true three-dimensional imaging of biological nanostructures and processes at cellular resolution that MRI can provide. On the other hand, MRI has a low sensitivity that can be compensated by the high sensitivity offered by fluorescence optical imaging (1). Thus, fluorescein-loaded iron (III) oxide nanoparticles may potentially be applied as multimodal nanoparticles by using its superparamagnetic iron (III) ions and fluorescein to generate MRI and fluorescence signals respectively for simultaneous detection. Previously, oleic acid-pluronic-coated iron (III) oxide nanoparticles loaded with indomethacin were developed in our laboratory (2). In this study, hydrophobic fluorescein was loaded instead of indomethacin onto the hydrophobic oleic acid layer coating the pluronic-iron oxide nanoparticles with the composition of 23.0 wt. % of oleic acid and 20.4 wt. % of pluronic.

Experimental methods

The optimum amount of oleic acid (23.0 wt. % of oleic acid) was used to coat 30 mg of the iron oxide nanoparticles. The optimum amount of pluronic (20.4 wt. % of pluronic) was used to coat 38.95 mg of the oleic acid-coated nanoparticles (23.0 wt. % of oleic acid). Different weight percentages (0 wt. %, 4.1 wt. %, 8.2 wt. %, 12.3 wt. %, 14.3 wt. % and 16.3 wt. %) of fluorescein were added to 48.95 mg of the oleic acid-pluronic-coated nanoparticles (23.0 wt. % of oleic acid and 20.4 wt. % of pluronic). The coated nanoparticles were characterized by FT-IR spectroscopy, zetasizer and fluorescence microscopy. The dispersion stability of the coated nanoparticles was also determined.

Results and discussion

The optimum formulation of fluoresein-loaded and oleic acid-pluronic-coated nanoparticles (23.0 wt. % of oleic acid, 20.4 wt. % of pluronic and 12.3 wt. % of fluorescein) was developed (Figure 1).

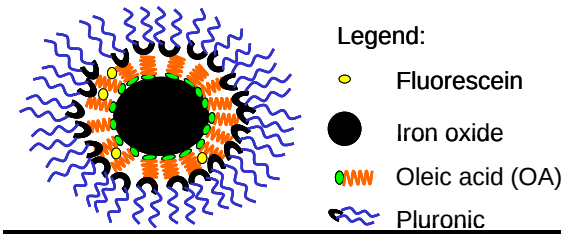

Figure 1. Diagram of a fluoresein-loaded and oleic acid-pluronic-coated nanoparticles (23.0 wt. % of oleic acid, 20.4 wt. % of pluronic and 12.3 wt. % of fluorescein).

It was verified by FT-IR spectral analysis that the oleic acid coating and fluorescein were present in the formulation. The aqueous dispersion of the formulation showed that the hydrophilic outer pluronic coating was present in addition to the oleic acid coating. The mean particle size measured by zetasizer and fluorescence microscope was 201.47 ± 10.8 nm and 13 ± 2 nm respectively (Figure 2).

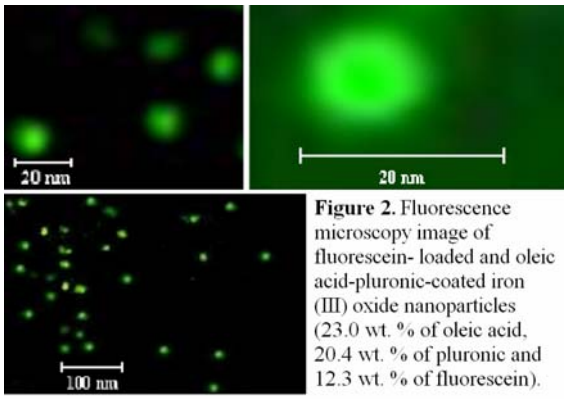

Figure 2. Fluorescence microscopy image of fluorescein- loaded and oleic acid-pluronic-coated iron (III) oxide nanoparticles (23.0 wt. % of oleic acid, 20.4 wt. % of pluronic and 12.3 wt. % of fluorescein).

The negative magnitude of the zeta potential (– 7.13 ± 0.06 mV) measured by zetasizer, coupled with the stable aqueous dispersity of the particles discourage nanoparticle aggregation and thus emboli formation on injection, which is crucial for potential *in vivo* application. The high optimum fluorescein loading capacity (116.3 ± 2.1 µg of fluorescein / mg of coated nanoparticles formulation) and the low percentage of fluorescein that leached from the formulation on production (5.2 % to 6.2 %) showed the potential application of the nanoparticles as multimodal nanoparticles to generate an intense fluorescence signal. The entrapment of fluorescein in the inner oleic acid layer and the poor water solubility of fluorescein of not more than 1.0% could also potentially shield fluorescein from leaching into the *in vivo* aqueous environment. Fluorescein entrapment could also potentially minimise fluorescein exposure to light, oxygen and other chemicals in the body that were involved in its photodegradation, thereby increasing its plasma half-life and fluorescence signal.

Conclusion

Further investigations are required to determine if the fluorescein-loaded and oleic acid-pluronic-coated iron (III) oxide nanoparticles are suitable for application as multimodal nanoparticles. Firstly, transmission electron microscope studies are needed to establish if the particles have a small mean diameter (< 50 nm), which is essential in *in vivo* applications: firstly, to discourage nanoparticle aggregation and thus prevent emboli formation on injection; and secondly, to discourage the removal of the nanoparticles by the reticuloendothelial system and thus prolong its plasma half-life. Secondly, it needs to be further determined if the magnetisation values of the formulated nanoparticles are sufficiently high after surface modification to generate a strong MRI signal. Thirdly, further *in vivo* animal studies are needed to ascertain if *in vivo* human applications of these particles are possible. Fourthly, the production of the particles needs to be improved to yield a polydispersity index of < 0.150 to achieve a satisfactory narrow particle size distribution. In addition, it needs to be further determined if the oleic acid-pluronic coating allows a targetable delivery with particle localization in a specific part of the body.

References

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