UNIVERSITI PUTRA MALAYSIA

LIPID NANOPARTICLES IN ANTI-BREAST CANCER DRUG DELIVERY SYSTEMS AND DRUG-MEMBRANE INTERACTIONS

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FPV 2014 12

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By

HOW CHEE WUN

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

July 2014

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

LIPID NANOPARTICLES IN ANTI-BREAST CANCER DRUG DELIVERY SYSTEMS AND DRUG-MEMBRANE INTERACTIONS

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July 2014

Chairman: Rasedee Abdullah, PhD
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Current anticancer drugs are plagued with lack of sustained effect and poor delivery. Many current studies focus on the use of drug carriers, particularly lipid nanoparticles as new drug delivery systems. In this study, a nanostructured lipid carrier (NLC) was formulated to serve as a carrier for tamoxifen (TAM). The study is also undertaken to determine the drug-membrane interaction through the use of liposomes as a membrane model. Hence, the main objectives of this study are to develop and determine the physicochemical and biological properties of NLC loaded with TAM (TAM-NLC), and to determine the interaction between the trimethoxybenzoyl analogue of catechin gallate (TMCG) and lipid membrane.

The NLC and TAM-NLC were prepared by high pressure homogenisation method. The lipid phase consisted of hydrogenated palm oil, olive oil and phosphatidylcholine as the lipid phase, while the aqueous phases are polysorbate 80, sorbitol, thimerosal and double-distilled water. The major components in the formulation were carefully chosen based on the absence of cytotoxicity towards a normal cell line (murine fibroblasts, BALB/c 3T3). The physicochemical characteristics of NLC, i.e. particle size, zeta potential (ZP), thermal profile, crystallinity, morphology and stability were assessed by photon correlation spectroscopy (PCS), laser doppler velocimetry, differential scanning calorimetry (DSC), wide-angle X-ray diffractometry (WAXD), transmission electron microscopy (TEM) and spectrophotometry, respectively. The release kinetics of TAM-NLC was determined by the Franz diffusion cell system while its cytotoxicity was determined *in vitro* on the human breast (MCF-7) and mouse mammary (4T1) cancer cell lines. To determine the drug-membrane interaction, a dried lipid film of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) was used to form liposomes and to entrap TMCG and quinone methide (QM, metabolite of TMCG) separately. The interaction of drug with the model membrane was assessed by DSC, WAXD, small-angle X-ray diffractometry (SAXD) and Fourier-transform infrared (FTIR). The PyMOL software was used to construct the molecular model of drug and membrane interaction.

The study showed that hydrogenated palm oil, trilaurin and docosanoic acid were significantly less cytotoxic than palmitin. Since surfactants influenced the physicochemical properties of the NLC, polysorbate 20 and 80 were assessed for use in the NLC formulation. The NLC formulated with polysorbate 80 showed good compatibility with the lipid phase, while polysorbate 20 caused destabilisation of the nanoparticles that resulted in phase separation during storage. With polysorbate 80 as surfactant, the NLCs are relatively spherical, with an average size of 102.8 nm, zeta potential of -30.57 mV, and possessed superior particle surface area to volume ratios. The transition temperature of NLC formulated with polysorbate 80 was 55.85 °C, which was lower than that formulated with polysorbate 20 or unprocessed lipid. The results indicated that NLC formulated with polysorbate 80 is of lower crystallinity and this was confirmed by WAXD. The NLC was also shown to be of low cytotoxicity to BALB/c 3T3 cell line. The NLC incubated with foetal bovine serum-supplemented media did not show increase in particle size, suggesting that its stability is good and practicality for use in intravenous administration. The stability of TAM-NLC was determined by storage at physiological pHs. The formulation is more stable at pH 7.4 (blood pH) even though its ZP was lower compared to pH 2.3 (stomach pH). The release of TAM from TAM-NLC followed first-order kinetics, while showing high cytotoxicity to MCF-7 and 4T1 cell lines with half-minimal inhibitory concentration of 5.56 and 5.19 μg mL^{-1}, respectively.

To determine the drug-membrane interaction, TMCG was used as the prodrug model and liposomes as the cell membrane model. The DSC analysis showed that TMCG was incorporated into DPPC membranes and had intercalated in-between the phospholipids molecules while reducing the cooperativity and lowering the transition temperature of the gel to liquid-crystalline phase. In addition, TMCG did not affect the macroscopic bilayer organisation of the liposomes; instead it decreased the thickness of the bilayer by forming an interdigitated gel phase. Quinone methide, the active form of TMCG however, showed limited interaction with the phospholipid bilayer indicating that a superficial interaction had occurred between QM and the phospholipid membrane with a weak gel stabilising effect and decreased hydrogen-bonding pattern of the interfacial region of the phospholipid. These results concur with the molecular dynamics simulation studies, which showed that TMCG was incorporated into the membrane phospholipid palisade while QM was excluded and interacted weakly with the polar portion of the lipid bilayer.

In conclusion, the study showed that the optimised NLC formulation with low cytotoxicity is a superior vehicle for encapsulation and carriage of TAM. The TAM-NLC developed in this study showed controlled-released characteristics, good stability at physiological pH with potential for tumour targeting. The study also reinforced the reliability of liposomes as cell membrane models, and TMCG interacts very well with it.

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

NANOZARAH LIPID DALAM SISTEM PENGHANTARAN DRUG ANTI-KANSER PAYUDARA DAN INTERAKSI DRUG-MEMBRAN

Oleh

HOW CHEE WUN

Julai 2014

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Drug anti-kanser semasa dibelenggu dengan kekurangan kesan berterusan dan penghantaran yang lemah. Banyak kajian kini menumpu kepada penggunaan pembawa drug, terutamanya nanozarah lipid sebagai sistem penghantar drug baharu. Dalam kajian ini, pembawa lipid nanostruktur (NLC) telah dirumuskan sebagai pembawa kepada tamoxifen (TAM). Kajian ini juga dijalankan untuk menentukan interaksi drug-membran melalui penggunaan liposom sebagai model membran. Oleh itu, objektif utama kajian ini adalah untuk mengembang dan menentukan sifat fizikokimia dan biologi NLC yang dimuatkan dengan TAM (TAM-NLC) serta menentukan interaksi antara analog trimetoksibenzoil katekin galat (TMCG) dengan membran lipid.

Pembawa lipid nanostruktur dan TAM-NLC telah disediakan melalui kaedah penghomogenan tekanan tinggi. Fasa lipid mengandungi minyak sawit terhidrogen, minyak zaitun, dan fosfatidilkolina sebagai fasa lipid, manakala fasa akueus adalah polisorbat 80, sorbitol, timerosal, dan air suling berganda. Komponen utama dalam rumusan ini telah dipilih dengan teliti berdasarkan ketiadaan kesitotoksikan terhadap sel normal (fibroblas murin, BALB/c 3T3). Ciri fizikokimia NLC, iaitu saiz zarah, potensi zeta (ZP), profil terma, kehabluran, morfologi, dan kestabilan telah dinilai melalui spektroskopi korelasi foton (PCS), velosimetri laser Doppler, kalorimetri imbasan pembezaan (DSC), difraktometri sinar-X sudut lebar (WAXD), mikroskopi elektron pancaran (TEM), dan spektrofotometri. Kinetik pembebasan TAM-NLC telah ditentukan melalui sistem sel resapan Franz manakala kesitotoksikannya ditentukan secara *in vitro* pada titisan sel kanser payudara manusia (MCF-7) dan mama mencit (4T1). Untuk menentukan interaksi drug-membran, filem lipid kering untuk 1,2-dipalmitoil-*sn*-glisero-3-fosfokolina (DPPC) telah digunakan untuk membentuk liposom dan memerangkap TMCG dan kuinon metida (QM, metabolit TMCG) secara berasingan. Interaksi drug dengan membran model dinilai melalui DSC, WAXD, difraktometri sinar-X sudut kecil (SAXD), dan inframerah Fourier-jelmaan. Perisian PyMOL telah digunakan untuk membina model molekul interaksi drug dengan membran.

Kajian itu menunjukkan bahawa kesitotoksikan minyak sawit terhidrogen, trilaurin dan asid dokosanoik adalah kurang tererti berbanding palmitin. Memandangkan surfaktan mempengaruhi sifat fizikokimia NLC, polisorbat 20 dan 80 telah dinilai untuk digunakan dalam rumusan NLC. Pembawa lipid nanostruktur yang dirumuskan dengan polisorbat 80 menunjukkan keserasian baik dengan fasa lipid, manakala polisorbat 20 menyebabkan ketakstabilan nanozarah yang mengakibatkan pemisahan fasa semasa penyimpanan. Dengan polisorbat 80 sebagai surfaktan, NLC mempunyai bentuk yang agak sfera, purata saiz 102.8 nm, potensi zeta -30.57 mV, dan mempunyai kawasan nisbah permukaan zarah kepada isipadu yang lebih tinggi. Suhu peralihan NLC yang dirumuskan dengan polisorbat 80 adalah 55.85 °C, iaitu lebih rendah daripada yang dirumuskan dengan polisorbat 20 atau lipid belum diproses. Keputusan kajian menunjukkan bahawa NLC dirumuskan dengan polisorbat 80 lebih rendah kehablurannya dan ini telah disahkan melalui WAXD. Pembawa lipid nanostruktur juga menunjukkan kesitotoksikan rendah terhadap titisan sel BALB/c 3T3. Pembawa lipid nanostruktur apabila dieram dalam media yang ditambah dengan serum bovin fetus tidak menunjukkan peningkatan dalam saiz zarah dan ini menyarankan yang kestabilannya adalah baik and sesuai untuk diguna dalam pemberian intravena. Kestabilan TAM-NLC telah ditentukan melalui penyimpanan pada pH fisiologi. Rumusan ini adalah lebih stabil pada pH 7.4 (pH darah) walaupun ZPnya lebih rendah berbanding pada pH 2.3 (pH perut). Pembebasan TAM daripada NLC mengikuti kinetik tertib pertama, sambil menunjukkan kesitotoksikan yang tinggi terhadap titisan sel MCF-7 dan 4T1 dengan kepekatan perencatan separuh minimum masing-masing 5.56 dan 5.19 μg mL^{-1}.

Untuk menentukan interaksi drug-membran TMCG diguna sebagai model prodrug dan liposom sebagai model membran sel. Analisis DSC menunjukkan TMCG telah tersebati dalam membran DPPC dan telah tersaling selit di antara molekul fosfolipid sambil mengurangkan kekerjasamaannya dan merendahkan suhu peralihan fasa gel kepada cecair-hablur. Di samping itu, TMCG tidak menjejaskan organisasi dwilapisan makroskopi liposom; sebaliknya ia mengurangkan ketebalan dwilapisan dengan membentuk fasa gel terinterdigit. Kuinone metida, iaitu bentuk aktif TMCG bagaimanapun berinteraksi secara terhad dengan dwilapisan fosfolipid, menunjukkan bahawa telah berlaku interaksi superfisial antara QM dan membran fosfolipid dengan kesan penstabilan gel lemah dan pengurangan pola ikatan hidrogen pada kawasan intermuka fosfolipid. Keputusan kajian ini selaras dengan kajian simulasi dinamik molekul, yang menunjukkan bahawa TMCG telah tersebati dalam deretan fosfolipid membran sambil QM pula terkeluar daripada dwilapisan ini dan berinteraksi secara lemah dengan bahagian khutub dwilapisan tersebut.

Kesimpulannya, kajian ini menunjukkan bahawa rumusan NLC yang dioptimumkan kepada kesitotoksikan rendah adalah pembawa yang unggul untuk pengkapsulan dan penghantaran TAM. The TAM-NLC yang dikembangkan dalam kajian ini menunjukkan ciri pembebasan berpanjangan, kestabilan yang baik pada pH fisiologi dan berpotensi dalam penyasaran

tumor. Kajian ini juga menunjukkan bahawa liposom adalah sebuah model membran sel yang sesuai, dan berinteraksi secara baiknya dengan TMCG.

ACKNOWLEDGEMENTS

Universiti Putra Malaysia has provided me with invaluable experiences. I am indebted to my supervisor, Prof. Dr Rasedee Abdullah, for his guidance and affectionate encouragement throughout the study. My appreciation is also directed to the co-supervisory committee, Prof. Dr Sivakumar Manickam and Prof. Dr Rozita Rosli, for their assistance and professional comments during the course of this study. I also dedicate my heart-felt acknowledgement to Prof. Dr Jose Neptuno Rodríguez-López, Prof. Dr Francisco Jose Aranda, Prof. Dr Francisco García-Cánovas and Prof. Dr Antonio Ortiz of Departamento de Bioquimica y Biología Molecular-A, Facultad de Veterinaria, Universidad de Murcia, Spain, for their unselfish knowledge-sharing. It is a great honour for me to have met and worked with these great professors.

My thanks to the entire staff and graduate students in the Laboratory of Vaccines and Immunotherapeutics (LIVES) and Departamento de Bioquímica y Biología Molecular-A, for companionship, and providing research facilities and assistance directly or indirectly for the past few years, particularly Dr Teo Guan Young, Dr Nagi Al-Haj, Mr Ng Wei Keat, Ms Wong Mun Theng, Mr Foo Jhi Biau, Dr Latifah Saiful Yasan, Dr Heshu Sulaiman Rahman, Mdm Norhazalina Md. Isa, Mdm Nancy Liew Woan Charn, Ms Arba'ah Md. Salleh, Dr Ng Hui Suan, Dr Soledad Chazarra, Dr María Fernanda Montenegro, Dr María Piedad Fernández-Pérez and Ms María del Mar Collado-González.

The study funded by the Ministry of Science, Technology and Innovation (MOSTI), Graduate Research Fellowship (GRF), MyBrain15 Scholarship and MOVER Mobility are highly valued.

The study would not be completed without the patience and perpetual support of my family members. They are the source of my inspiration and motivation. Last but not least, my sincere gratitude is extended to Ms Chong Hui Syn and Ms Loo Yuet Chee for their understanding.

I certify that a Thesis Examination Committee has met on 21 July 2014 to conduct the final examination of How Chee Wun on his thesis entitled “Lipid Nanoparticles in Anti-Breast Cancer Drug Delivery Systems and Drug-Membrane Interactions” in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A)106] 15 March 1998. The Committee recommends that the student be awarded the degree of Doctor of Philosophy.

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

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
A_{spec}	Specific surface area
BSA	Bovine serum albumin
CI	Crystallinity index
DHFR	Dihydrofolate reductase
DL	Drug loading capacity
DLS	Dynamic light scattering
DMSO	Dimethyl sulfoxide
DPPC	Dipalmitoyl-phosphatidylcholine
DSC	Differential scanning calorimetry
EDTA	Ethylenediaminetetraacetic acid
EE	Entrapment efficiency
e.g.	Exempli gratia
EGCG	(–)-epigallocatechin gallate
ER	Oestrogen receptor
ER^+	Oestrogen receptor positive
etc.	Et cetera
FBS	Foetal bovine serum
FDA	US Food and Drug Administration
FFF	Field fractionation
FTIR	Fourier-transform infrared spectroscopy
GAP	Global analysis program
GRAS	Generally Recognised as Safe
HEPES	4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid
HLB	Hydrophilic to lipophilic balance
HPH	High pressure homogenisation
HPLC	High performance liquid chromatography
HPO	Hydrogenated palm oil
h	Hour(s)
i.e.	Id est
IR	Infrared
LD	Laser diffraction
LDV	Laser doppler velocimetry
MCG	Modified Caille-Gauss
MD	Molecular dynamics simulation
MPS	Mononuclear phagocyte system
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NCI	National Cancer Institute
NIBS	Non-invasive back scatter
NIEHS	Institute of Environmental Health Sciences
NIH	National Institute of Health
NLC	Nanostructured lipid carriers
NLC20	Polysorbate 20-stabilised nanostructured lipid carriers
NLC80	Polysorbate 80-stabilised nanostructured lipid carriers
NLC_m	Nanostructured lipid carriers in serum-free medium
NLC_{sm}	Nanostructured lipid carriers in serum-supplemented medium
NMR	Nuclear magnetic resonance

OD	Optical density
OR	Ostwald's ripening
ρ	Density
PBS	Phosphate buffer saline
PC	L-α-phosphatidylcholine
PCS	Photon correlation spectroscopy
PDI	Polydispersity index
PEG	Polyethylene glycol
PES	Polyethersulfone
PGA	Polyglycolic acid
PLA	Polylactic acid
PLGA	Poly(lactide-*co*-glycolide) acid
PN	Polymeric nanoparticles
PR	Progesterone receptor
PTA	Phosphotungstic acid
QM	Quinone methide
r	Radius
RES	Reticuloendothelial system
rpm	Rotation per minute
rt	Room temperature
SAXD	Small-angle powder diffraction
SDS	Sodium dodecyl sulphate
SEE	Solvent emulsification-evaporation
SEM	Scanning electron microscopy
SERM	Selective oestrogen receptor modulator
SLN	Solid lipid nanoparticles
SPC	Single point charge
TAM	Tamoxifen
TAM-NLC	Tamoxifen-loaded nanostructured lipid carriers
TEM	Transmission electron microscopy
TMCG	3-O-(3,4,5-trimethoxybenzoyl)-(–)-catechin gallate
TMECG	3-O-(3,4,5-trimethoxybenzoyl)-(–)-epicatechin gallate
UV-VIS	Ultra violet-visible light
W	Weight
WAXD	Wide-angle X-ray diffraction
XRD	X-ray diffractometry
ZP	Zeta potential

CHAPTER 1

INTRODUCTION

Cancers are feared diseases because of the impact they have on the patient. Individuals with cancers either die prematurely or continue to survive with a poor quality of life. In women one of the most frequent cancers is breast cancer, affecting one in eight women. This disease is the second leading cause of cancer deaths in women after lung cancer (Siegel *et al.*, 2011). Worldwide, over one million new cancer cases are diagnosed each year and among these patients, 400 thousand die from breast cancer (Coughlin and Ekwueme, 2009). In Malaysia, one in 19 women is at risk of developing the cancer (Ferlay *et al.*, 2010).

The main goal of chemotherapeutic regimen is to increase the therapeutic index; however the overwhelming resistance of the tumour cell to therapy and undesirable side effects of drugs to a certain extent offset the benefits (Ewesuedo and Ratain, 2003). These drugs, despite producing significant effects on cancers, are rarely effective in the restoration of full health. Instead they ameliorate the condition of the patients. Among the limitations faced by most conventional drugs is the inability to deliver appropriate amounts to the target. Current chemotherapeutics are also plagued with problems of low solubility and thus exhibiting low bioavailability after administration. It is estimated that of 40% of drugs in the pipelines are poorly soluble and approximately 60% of these therapeutic compounds are synthesised (Merisko-Liversidge *et al.*, 2003). The poor solubility of these compounds dramatically reduces their efficacy as an anticancer drug. Thus it is becoming more apparent that development of new drugs alone is not sufficient to ensure their utility in cancer therapy.

Chemotherapeutic agents are usually biodistributed to the entire body, resulting in high drug concentrations in plasma and non-targeted sites inherently causing adverse effects. These problems can be resolved by two approaches, i.e. development of a new chemical entity that possess better specificity to target cancerous cell, and discovery of new strategies to overcome the poor drug efficacy. The first approach has been shown to have a limited success, as clearly demonstrated by the slow progress in the discovery of these drugs. The new therapeutic approach however, poses questions and challenges to not only the development of new treatment methods, but also the mechanisms by which the drugs are to be delivered. Among the strategies adopted is chemical modification of drug molecules to prodrugs and drug encapsulation into suitable delivery systems. Both strategies have been shown to have great potential in curbing cancers.

Innovative drug delivery systems, like nanoparticle carrier systems, have been claimed to improve the pharmacological properties of various active ingredients, resulting in an increased drug circulation time and enhanced efficiency (Lee, 2006). These effects are often due to the altered

pharmacokinetics and biodistribution of the drugs upon encapsulation into specific drug carriers. Since these carriers are nano-sized drug delivery vehicles, they can confer drug targeting and ease of intravenous administration without the risk of embolisation (Cullen, 2001).

Nanoscience is an interdisciplinary field that has its early beginning in 1980s. In medicine, nanoparticles as drug carriers are showing to have vast potentials. Among the nanocarrier systems for drug delivery are the colloids (Sharma *et al.*, 2010). Colloidal carriers include nanosuspensions, nanoemulsions, polymeric nanoparticles, liposomes and nanostructured lipid carriers (NLC) are versatile in their formulation and production for therapeutic purposes. Among the advantages of the colloid drug carriers are sustained drug release characteristics and effectiveness in the delivery active ingredients to target organs and tissues. Hence, it can increase therapeutic effects while minimising side effects (Wagner *et al.*, 2006).

The versatility of colloids in oncology was not only demonstrated by their application in nanomedicine, but also by drug-membrane interactions. The liposomes are often used as biological membrane models to determine the drug-membrane interactions. Since new drugs are being discovered every year, which have diverse structures, their capability to cross or bind to lipid membranes must be determined before its utility as therapeutic compound can be ascertained. The information will provide better understanding of the mechanism by which the drugs interact with cell membranes at molecular level and predict their pharmacological activities and effect on the biological processes.

In this study NLC was used as a carrier for tamoxifen (TAM), an anti-breast cancer drug. The NLC was optimised before loaded with TAM. The physiochemical characteristics and therapeutic effect of the tamoxifen-loaded NLC (TAM-NLC) on breast cancer cell lines were determined. The objectives of this study are to develop TAM-NLC and to determine its physical and anti-cancer properties.

The second part of the study determined the drug-membrane interaction using liposomes as the membrane model. Apart from reinforcing the usefulness of liposomes in oncology, the objective of the second study is to confirm the membrane perturbing properties of novel prodrug 3-O-(3,4,5-trimethoxybenzoyl)-(–)-catechin (TMCG) has on theoretical membrane. To achieve this objective, the effect of TMCG and its activated product, quinone methide (QM), on the thermotropic and structural properties of phosphatidylcholine (the most important phospholipid in eukaryotic membranes) membrane was also determined.

1.1 Hypotheses

1. The optimised NLC can achieve a size less than 100 nm.
2. Tamoxifen can incorporate into NLC with good stability.
3. The activity of TAM retains even it was subjected to harsh processes.
4. The TMCG interacts very well with dipalmitoyl-phosphatidylcholine (DPPC) membrane.
5. Quinone methide does not interact readily with the DPPC membrane.

1.2 Objectives

The objectives of the study were to

1. develop and determine the physicochemical properties of NLC.
2. determine the characteristics and cytotoxic effect of NLC.
3. develop and characterise TAM-NLC.
4. assess the stability and cytotoxic effect of TAM-NLC.
5. determine the effect of TMCG and QM on structural properties of DPPC membranes.

REFERENCES

Abbasalipourkabir, R., Salehzadeh, A., and Abdullah, R. (2010). Antitumour activity of tamoxifen loaded solid lipid nanoparticles on induced mammary tumour gland in Sprague-Dawley rats. *African Journal of Biotechnology, 9*(43), 7337-7345.

Abbasalipourkabir, R., Salehzadeh, A., and Abdullah, R. (2011). Delivering tamoxifen within solid lipid nanoparticles. *Pharmaceutical Technology, 35*(4), 74-79.

Adachi, S., Nagao, T., Ingolfsson, H. I., Maxfield, F. R., Andersen, O. S., Kopelovich, L., and Weinstein, I. B. (2007). The inhibitory effect of (–)-epigallocatechin gallate on activation of the epidermal growth factor receptor is associated with altered lipid order in HT29 colon cancer cells. *Cancer Research, 67*(13), 6493-6501.

Agrawal, Y., Petkar, K. C., and Sawant, K. K. (2010). Development, evaluation and clinical studies of Acitretin loaded nanostructured lipid carriers for topical treatment of psoriasis. *International Journal of Pharmaceutics, 401*(1), 93-102.

Ahmed, F., Pakunlu, R. I., Srinivas, G., Brannan, A., Bates, F., Klein, M. L., Minko, T., and Discher, D. E. (2006). Shrinkage of a rapidly growing tumour by drug-loaded polymersomes: pH-triggered release through copolymer degradation. *Molecular Pharmaceutics, 3*(3), 340-350.

Al-Haj, N. A., Abdullah, R., Ibrahim, S., and Bustamam, A. (2008). Tamoxifen drug loading solid lipid nanoparticles prepared by hot high pressure homogenisation techniques. *American Journal of Pharmacology and Toxicology, 3*(3), 219.

Ali, H., El-Sayed, K., Sylvester, P. W., and Nazzal, S. (2010). Molecular interaction and localisation of tocotrienol-rich fraction (TRF) within the matrices of lipid nanoparticles: Evidence studies by differential scanning calorimetry (DSC) and proton nuclear magnetic resonance spectroscopy (^{1}H NMR). *Colloids and Surfaces B: Biointerfaces, 77*(2), 286-297.

Alkner, S., Bendahl, P. O., Fernö, M., Nordenskjöld, B., and Rydén, L. (2009). Tamoxifen reduces the risk of contralateral breast cancer in premenopausal women: Results from a controlled randomised trial. *European Journal of Cancer, 45*(14), 2496-2502.

Almeida, A. J., and Souto, E. (2007). Solid lipid nanoparticles as a drug delivery system for peptides and proteins. *Advanced Drug Delivery Reviews, 59*(6), 478-490.

Amaral, C., Borges, M., Melo, S., da Silva, E. T., Correia-da-Silva, G., and Teixeira, N. (2012). Apoptosis and autophagy in breast cancer cells following Exemestane treatment. *PLoS One, 7*(8), e42398.

Amelinckx, S., van Dyck, D., van Landuyt, J., and van Tendeloo, G. (2008). *Handbook of Microscopy: Applications in Materials Science, Solid-State Physics, and Chemistry. Methods II* (Volume 2): Wiley-VCH.

Andersen, O. S., and Koeppe, R. E. (2007). Bilayer thickness and membrane protein function: An energetic perspective. *Annual Review of Biophysical and Biomolecular Structures, 36*, 107-130.

Ascierto, P. A., Streicher, H. Z., and Sznol, M. (2010). Commentary Melanoma: A model for testing new agents in combination therapies. *Journal of Translational Medicine, 8*(38), 1-7.

Avgoustakis, K. (2004). Pegylated poly(lactide) and poly(lactide-co-glycolide) nanoparticles: Preparation, properties and possible applications in drug delivery. *Current Drug Delivery, 1*(4), 321-333.

Babich, H., and Visioli, F. (2003). *In vitro* cytotoxicity to human cells in culture of some phenolics from olive oil. *Il Farmaco, 58*(5), 403-407.

Babich, H., Zuckerbraun, H. L., and Weinerman, S. M. (2007). *In vitro* cytotoxicity of (–)-catechin gallate, a minor polyphenol in green tea. *Toxicology Letters, 171*(3), 171-180.

Bakshi, M. S., Kaura, A., Kaur, G., Torigoe, K., and Esumi, K. (2006). Effect of sodium dodecylsulfate and dodecyltrimethyl ammonium bromide on the morphologies of gold nanoparticles in the presence of poly (amidoamine) dendrimers. *Journal of Nanoscience and Nanotechnology, 6*(3), 644-650.

Balakrishnan, A., Rege, B. D., Amidon, G. L., and Polli, J. E. (2004). Surfactant-mediated dissolution: Contributions of solubility enhancement and relatively low micelle diffusivity. *Journal of Pharmaceutical Sciences, 93*(8), 2064-2075.

Barratt, G. M. (2000). Therapeutic applications of colloidal drug carriers. *Pharmaceutical Science and Technology Today, 3*(5), 163-171.

Bastian, S., Busch, W., Springer, A., Meißner, T., Holke, R., Scholz, S., Iwe, M., Pompe, W., Gelinsky, M., and Potthof, A. (2008). Toxicity of tungsten carbide and cobalt-doped tungsten carbide nanoparticles in mammalian cells *in vitro*. *Environmental Health Perspectives, 117*(4), 530-536.

Berendsen, H. J. C., Postma, J. P. M., van Gunsteren, W. F., DiNola, A., and Haak, J. R. (1984). Molecular dynamics with coupling to an external bath. *The Journal of Chemical Physics, 81*, 3684.

Berendsen, H. J. C., Postma, J. P. M., Van Gunsteren, W. F., and Hermans, J. (1981). Interaction models for water in relation to protein hydration. *Intermolecular Forces, 11*(1), 331-342.

Berényi, S., Mihály, J., Kristyán, S., Nagy, L. N., Telegdi, J., and Bóta, A. (2013). Thermotropic and structural effects of poly (malic acid) on fully hydrated multilamellar DPPC-water systems. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, *1828*(2), 661-669.

Berg, J. M., Romoser, A., Banerjee, N., Zebda, R., and Sayes, C. M. (2009). The relationship between pH and zeta potential of 30 nm metal oxide nanoparticle suspensions relevant to *in vitro* toxicological evaluations. *Nanotoxicology, 3*(4), 276-283.

Bergenståhl, B. (2008). Physicochemical aspects of an emulsifier functionality. *Food Emulsifiers and Their Applications* (pp. 173-194): Springer.

Biswas, S., Dodwadkar, N. S., Deshpande, P. P., and Torchilin, V. P. (2012). Liposomes loaded with paclitaxel and modified with novel triphenylphosphonium-PEG-PE conjugate possess low toxicity, target mitochondria and demonstrate enhanced antitumour effects *in vitro* and *in vivo*. *Journal of Controlled Release, 159*(3), 393-402.

Blume, A., Hübner, W., and Messner, G. (1988). Fourier transform infrared spectroscopy of $^{13}C=O$ labeled phospholipids hydrogen bonding to carbonyl groups. *Biochemistry, 27*(21), 8239-8249.

Bohren, C. F., and Huffman, D. R. (2008). *Absorption and scattering of light by small particles*: John Wiley and Sons.

Böttcher, C. J. F., and Pries, C. (1961). A rapid and sensitive sub-micro phosphorus determination. *Analytica Chimica Acta, 24*, 203-204.

Budhian, A., Siegel, S. J., and Winey, K. I. (2007). Haloperidol-loaded PLGA nanoparticles: Systematic study of particle size and drug content. *International Journal of Pharmaceutics, 336*(2), 367-375.

Bunjes, H., and Koch, M. H. J. (2005). Saturated phospholipids promote crystallisation but slow down polymorphic transitions in triglyceride nanoparticles. *Journal of Controlled Release, 107*(2), 229-243.

Burgess, D. J. (2006). Colloids and colloid drug delivery system *Encyclopedia of Pharmaceutical Technology (Third Edition)* (pp. 636-647). London: Informa Healthcare.

Burstein, H. J., and Griggs, J. J. (2010). Adjuvant hormonal therapy for early-stage breast cancer. *Surgical Oncology Clinics of North America, 19*(3), 639.

Cai, Z., Wang, Y., Zhu, L. J., and Liu, Z. Q. (2010). Nanocarriers: A general strategy for enhancement of oral bioavailability of poorly absorbed or pre-systemically metabolised drugs. *Current Drug Metabolism, 11*(2), 197-207.

Caturla, N., Vera-Samper, E., Villalaín, J., Mateo, C. R., and Micol, V. (2003). The relationship between the antioxidant and the antibacterial properties of galloylated catechins and the structure of phospholipid model membranes. *Free Radical Biology and Medicine, 34*(6), 648-662.

Cecv, G., and Marsh, D. (1978). Phospholipid bilayers: Physical Principles and Models. *Cell Biology*. New Jersey: Wiley.

Chen, K. G., Valencia, J. C., Lai, B., Zhang, G., Paterson, J. K., Rouzaud, F., Berens, W., Wincovitch, S. M., Garfield, S. H., and Leapman, R. D. (2006). Melanosomal sequestration of cytotoxic drugs contributes to the intractability of malignant melanomas. *Proceedings of the National Academy of Sciences, 103*(26), 9903-9907.

Chen, X., Young, T. J., Sarkari, M., Williams, R. O., and Johnston, K. P. (2002). Preparation of cyclosporine A nanoparticles by evaporative precipitation into aqueous solution. *International Journal of Pharmaceutics, 242*(1), 3-14.

Chicano, J. J., Ortiz, A., Teruel, J. A., and Aranda, F. J. (2001). Organotin compounds alter the physical organisation of phosphatidylcholine membranes. *Biochimica et Biophysica Acta (BBA)-Biomembranes, 1510*(1), 330-341.

Chinsriwongkul, A., Chareanputtakhun, P., Ngawhirunpat, T., Rojanarata, T., Sila-on, W., Ruktanonchai, U., and Opanasopit, P. (2012). Nanostructured lipid carriers (NLC) for parenteral delivery of an anticancer drug. *AAPS Pharmaceutical Science and Technology, 13*(1), 150-158.

Chiu, S. W., Pandit, S. A., Scott, H., and Jakobsson, E. (2009). An improved united atom force field for simulation of mixed lipid bilayers. *The Journal of Physical Chemistry B, 113*(9), 2748-2763.

Cho, K., Wang, X. U., Nie, S., and Shin, D. M. (2008). Therapeutic nanoparticles for drug delivery in cancer. *Clinical Cancer Research, 14*(5), 1310-1316.

Choi, B. H., Choi, J. S., Min, D. S., Yoon, S. H., Rhie, D. J., Jo, Y. H., Kim, M. S., and Hahn, S. J. (2001). Effects of (–)-epigallocatechin-3-gallate, the main component of green tea, on the cloned rat brain Kv1. 5 potassium channels. *Biochemical Pharmacology, 62*(5), 527-535.

Chou, D. K., Krishnamurthy, R., Randolph, T. W., Carpenter, J. F., and Manning, M. C. (2005). Effects of Tween 20® and Tween 80® on the stability of Albutropin during agitation. *Journal of Pharmaceutical Sciences, 94*(6), 1368-1381.

Chow, H. S., Hakim, I. A., Vining, D. R., Crowell, J. A., Ranger-Moore, J., Chew, W. M., Celaya, C. A., Rodney, S. R., Hara, Y., and Alberts, D. S. (2005). Effects of dosing condition on the oral bioavailability of green tea catechins after single-dose administration of Polyphenon E in healthy individuals. *Clinical Cancer Research, 11*(12), 4627-4633.

Chu, B., and Liu, T. (2000). Characterisation of nanoparticles by scattering techniques. *Journal of Nanoparticle Research, 2*(1), 29-41.

Chung, H. B., Hennig, B., Cho, B. H., and Darnell, B. E. (1998). Effect of the fat composition of a single meal on the composition and cytotoxic potencies of lipolytically-releasable free fatty acids in postprandial plasma. *Atherosclerosis, 141*(2), 321-332.

Coates, A. S., Colleoni, M., and Goldhirsch, A. (2012). Is adjuvant chemotherapy useful for women with luminal A breast cancer? *Journal of Clinical Oncology, 30*(12), 1260-1263.

Conrad, D. (2006). Tumour-seeking nanoparticles. *NCI Alliance for Nanotechnology in Cancer: Monthly Feature, September*.

Coughlin, S. S., and Ekwueme, D. U. (2009). Breast cancer as a global health concern. *Cancer Epidemiology, 33*(5), 315-318.

Couvreur, P., Barratt, G., Fattal, E., Legrand, P., and Vauthier, C. (2002). Nanocapsule technology: A review. *Critical Reviews in Therapeutic Drug Carrier Systems, 19*(2), 99.

Cui, Y., Oh, Y. J., Lim, J., Youn, M., Lee, I., Pak, H. K., Park, W., Jo, W., and Park, S. (2012). AFM study of the differential inhibitory effects of the green tea polyphenol (–)-epigallocatechin-3-gallate (EGCG) against Gram-positive and Gram-negative bacteria. *Food Microbiology, 29*(1), 80-87.

Cullen, T. V. (2001). Innovative pharmaceutical treatments require innovative method of administration. *Modern Drug Delivery, 4*(4), 49-50.

Danielli, J. F., and Davson, H. (1935). A contribution to the theory of permeability of thin films. *Journal of Cellular and Comparative Physiology, 5*(4), 495-508.

Daniels, R. (2005). Lipid nanoparticles used in skin care cosmetics - processes and associated benefits. Retrieved 8 October, 2012, from http://www.azonano.com/article.aspx?ArticleID=1245

Das, S., Ng, W. K., and Tan, R. B. H. (2012). Are nanostructured lipid carriers (NLCs) better than solid lipid nanoparticles (SLNs): Development, characterisations and comparative evaluations of clotrimazole-loaded SLNs and NLCs? *European Journal of Pharmaceutical Sciences, 47*(1), 139-151.

Dash, S., Murthy, P. N., Nath, L., and Chowdhury, P. (2010). Kinetic modeling on drug release from controlled drug delivery systems. *Acta Poloniae Pharmaceutica, 67*(3), 217-223.

Davies, C., Godwin, J., Gray, R., Clarke, M., Cutter, D., Darby, S., McGale, P., Pan, H., Taylor, C., and Wang, Y. (2011). Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: Patient-level meta-analysis of randomised trials. *Lancet, 378*(9793), 771-784.

Dennis, C. L., Jackson, A. J., Borchers, J. A., Hoopes, P. J., Strawbridge, R., Foreman, A. R., Van Lierop, J., Grüttner, C., and Ivkov, R. (2009). Nearly complete regression of tumours via collective behavior of magnetic nanoparticles in hyperthermia. *Nanotechnology, 20*(39), 395103.

Dingler, A., Blum, R. P., Niehus, H., Muller, R. H., and Gohla, S. (1999). Solid lipid nanoparticles (SLN^{TM}/ Lipopearls^{TM}) a pharmaceutical and cosmetic carrier for the application of vitamin E in dermal products. *Journal of Microencapsulation, 16*(6), 751-767.

Doktorovova, S., and Souto, E. B. (2009). Nanostructured lipid carrier-based hydrogel formulations for drug delivery: A comprehensive review. *Expert Opinion on Drug Delivery, 6*(2), 165-176.

Dowling, A. P. (2004). Development of nanotechnologies. *Materials Today, 7*(12), 30-35.

Dreicer, R., Bajorin, D. F., McLeod, D. G., Petrylak, D. P., and Moul, J. W. (2011). New Data, New Paradigms for Treating Prostate Cancer Patients VI: Novel Hormonal Therapy Approaches. *Urology, 78*(5), S494-S498.

Drummond, D. C., Meyer, O., Hong, K., Kirpotin, D. B., and Papahadjopoulos, D. (1999). Optimizing liposomes for delivery of chemotherapeutic agents to solid tumours. *Pharmacological Reviews, 51*(4), 691-744.

Dubes, A., Parrot-Lopez, H., Abdelwahed, W., Degobert, G., Fessi, H., Shahgaldian, P., and Coleman, A. W. (2003). Scanning electron microscopy and atomic force microscopy imaging of solid lipid nanoparticles derived from amphiphilic cyclodextrins. *European Journal of Pharmaceutics and Biopharmaceutics, 55*(3), 279-282.

Dukhin, A., and Parlia, S. (2012). Studying homogeneity and zeta potential of membranes using electroacoustics. *Journal of Membrane Science*.

Edlund, U., and Albertsson, A. C. (2003). Polyesters based on diacid monomers. *Advanced Drug Delivery Reviews, 55*(4), 585-609.

Ekambaram, P., Sathali, A. A. H., and Priyanka, K. (2012). Solid lipid nanoparticles: A review. *Scientific Reviews and Chemical Communications, 2*, 80-102.

El-Bayoumi, T. A., and Torchilin, V. P. (2009). Tumour-targeted nanomedicines: Enhanced antitumour efficacy *in vivo* of doxorubicin-loaded, long-circulating liposomes modified with cancer-specific monoclonal antibody. *Clinical Cancer Research, 15*(6), 1973-1980.

El-Sayed, M., Abdallah, O. Y., Naggar, V. F., and Khalafallah, N. M. (2007). PG-liposomes: Novel lipid vesicles for skin delivery of drugs. *Journal of Pharmacy and Pharmacology, 59*(10), 1447-1450.

Ernst, R., and Arditti, J. (1980). Biological effects of surfactants, IV. Effects of non-ionics and amphoterics on HeLa cells. *Toxicology, 15*(3), 233-242.

Essmann, U., Perera, L., Berkowitz, M. L., Darden, T., Lee, H., and Pedersen, L. G. (1995). A smooth particle mesh Ewald method. *Journal of Chemical Physics, 103*(19), 8577-8593.

Ewesuedo, R. B., and Ratain, M. J. (2003). Principles of cancer chemotherapy *Oncologic Therapies* (pp. 19-66): Springer.

Fang, J. Y., Fang, C. L., Liu, C. H., and Su, Y. H. (2008). Lipid nanoparticles as vehicles for topical psoralen delivery: Solid lipid nanoparticles (SLN) versus nanostructured lipid carriers (NLC). *European Journal of Pharmaceutics and Biopharmaceutics, 70*(2), 633-640.

Ferlay, J., Shin, H. R., Bray, F., Forman, D., Mathers, C., and Parkin, D. M. (2010). Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *International Journal of Cancer, 127*(12), 2893-2917.

Fernández-Pérez, M. P., Montenegro, M. F., Sáez-Ayala, M., Sánchez-del-Campo, L., Piñero-Madrona, A., Cabezas-Herrera, J., and Rodríguez-López, J. N. (2013). Suppression of antifolate resistance by targeting the myosin Va trafficking pathway in melanoma. *Neoplasia, 15*(7), 826.

Fisher, B., Costantino, J. P., Wickerham, D. L., Redmond, C. K., Kavanah, M., Cronin, W. M., Vogel, V., Robidoux, A., Dimitrov, N., and Atkins, J. (1998). Tamoxifen for prevention of breast cancer: Report of the National Surgical Adjuvant Breast and Bowel Project P-1 Study. *Journal of the National Cancer Institute, 90*(18), 1371-1388.

Fontana, G., Maniscalco, L., Schillaci, D., Cavallaro, G., and Giammona, G. (2005). Solid lipid nanoparticles containing tamoxifen characterisation and in vitro antitumoural activity. *Drug Delivery, 12*(6), 385-392.

Freitas, C., and Müller, R. H. (1999). Correlation between long-term stability of solid lipid nanoparticles (SLN™) and crystallinity of the lipid phase. *European Journal of Pharmaceutics and Biopharmaceutics, 47*(2), 125-132.

Fujiki, H., Imai, K., Nakachi, K., Shimizu, M., Moriwaki, H., and Suganuma, M. (2012). Challenging the effectiveness of green tea in primary and tertiary cancer prevention. *Journal of Cancer Research and Clinical Oncology, 138*(8), 1259-1270.

Fujiki, H., Suganuma, M., Imai, K., and Nakachi, K. (2002). Green tea: Cancer preventive beverage and/or drug. *Cancer Letters, 188*(1), 9-13.

Furuya, M., Yonemitsu, Y., and Aoki, I. (2009). III. Angiogenesis: Complexity of tumour vasculature and microenvironment. *Current Pharmaceutical Design, 15*(16), 1854-1867.

Gabizon, A., Horowitz, A. T., Goren, D., Tzemach, D., Mandelbaum-Shavit, F., Qazen, M. M., and Zalipsky, S. (1999). Targeting folate receptor with folate linked to extremities of poly(ethylene glycol)-grafted liposomes: *In vitro* studies. *Bioconjugate Chemistry, 10*(2), 289-298.

Gasco, M. R. (1993). Method for producing solid lipid microspheres having a narrow size distribution: Google Patents.

Gaumet, M., Gurny, R., and Delie, F. (2009). Localisation and quantification of biodegradable particles in an intestinal cell model: The influence of particle size. *European Journal of Pharmaceutical Sciences, 36*(4), 465-473.

Gaumet, M., Vargas, A., Gurny, R., and Delie, F. (2008). Nanoparticles for drug delivery: The need for precision in reporting particle size parameters. *European Journal of Pharmaceutics and Biopharmaceutics, 69*(1), 1-9.

Granovsky, A. A. (2012). Firefly version 8.0. 0.

Gulari, E., Gulari, E., Tsunashima, Y., and Chu, B. (2008). Photon correlation spectroscopy of particle distributions. *The Journal of Chemical Physics, 70*(8), 3965-3972.

Gullotti, E., and Yeo, Y. (2009). Extracellularly activated nanocarriers: A new paradigm of tumour targeted drug delivery. *Molecular Pharmaceutics, 6*(4), 1041-1051.

Guo, H. X., and Shi, Y. P. (2009). A novel zein-based dry coating tablet design for zero-order release. *International Journal of Pharmaceutics, 370*(1), 81-86.

Gupta, A. K., and Gupta, M. (2005). Synthesis and surface engineering of iron oxide nanoparticles for biomedical applications. *Biomaterials, 26*(18), 3995-4021.

Gupta, R. B. (2006). Fundamentals of Drug Nanoparticles. In G. R.B. and K. U.B. (Eds.), *Drugs and the Pharmaceutical Sciences: Nanoparticle Technology for Drug Delivery* (pp. 1-18). New York: Taylor and Francis.

Håkansson, A., Fuchs, L., Innings, F., Revstedt, J., Bergenståhl, B., and Trägårdh, C. (2010). Visual observations and acoustic measurements of cavitation in an experimental model of a high-pressure homogenizer. *Journal of Food Engineering, 100*(3), 504-513.

Han, Y. (2007). Synergic anticandidal effect of epigallocatechin-O-gallate combined with amphotericin B in a murine model of disseminated candidiasis and its anticandidal mechanism. *Biological and Pharmaceutical Bulletin, 30*(9), 1693-1696.

Hashimoto, T., Kumazawa, S., Nanjo, F., Hara, Y., and Nakayama, T. (1999). Interaction of tea catechins with lipid bilayers investigated with liposome systems. *Bioscience, Biotechnology, and Biochemistry, 63*(12), 2252-2255.

Hassan, M. A. (2007). A long acting ophthalmic gel formulations of atenolol. *Drug Development and Industrial Pharmacy, 33*(11), 1192-1198.

Hess, B., Bekker, H., Berendsen, H. J. C., and Fraaije, J. G. E. M. (1997). LINCS: A linear constraint solver for molecular simulations. *Journal of Computational Chemistry, 18*(12), 1463-1472.

Heuschkel, S., Goebel, A., and Neubert, R. H. H. (2008). Microemulsions: Modern colloidal carrier for dermal and transdermal drug delivery. *Journal of Pharmaceutical Sciences, 97*(2), 603-631.

Heydenreich, A. V., Westmeier, R., Pedersen, N., Poulsen, H. S., and Kristensen, H. G. (2003). Preparation and purification of cationic solid lipid nanospheres: Effects on particle size, physical stability and cell toxicity. *International Journal of Pharmaceutics, 254*(1), 83-87.

Hong, J., Lu, H., Meng, X., Ryu, J. H., Hara, Y., and Yang, C. S. (2002). Stability, cellular uptake, biotransformation, and efflux of tea polyphenol (–)-epigallocatechin-3-gallate in HT-29 human colon adenocarcinoma cells. *Cancer Research, 62*(24), 7241-7246.

Hou, D., Xie, C., Huang, K., and Zhu, C. (2003). The production and characteristics of solid lipid nanoparticles (SLNs). *Biomaterials, 24*(10), 1781-1785.

How, C. W., Rasedee, A., and Abbasalipourkabir, R. (2011). Physicochemical properties of nanostructured lipid carriers as colloidal carrier system stabilised with polysorbate 20 and polysorbate 80. *African Journal of Biotechnology, 10*(9), 1684-1689.

How, C. W., Rasedee, A., and Abbasalipourkabir, R. (2013). Characterisation and cytotoxicity of nanostructured lipid carriers formulated with olive oil, hydrogenated palm oil, and polysorbate 80. *NanoBioscience, IEEE Transactions on, 12*(2), 72-78.

Hu, F. Q., Jiang, S. P., Du, Y. Z., Yuan, H., Ye, Y. Q., and Zeng, S. (2005). Preparation and characterisation of stearic acid nanostructured lipid carriers by solvent diffusion method in an aqueous system. *Colloids and Surfaces B: Biointerfaces, 45*(3), 167-173.

Hu, F. Q., Jiang, S. P., Du, Y. Z., Yuan, H., Ye, Y. Q., and Zeng, S. (2006). Preparation and characteristics of monostearin nanostructured lipid carriers. *International Journal of Pharmaceutics, 314*(1), 83-89.

Hu, L., Tang, X., and Cui, F. (2004). Solid lipid nanoparticles (SLNs) to improve oral bioavailability of poorly soluble drugs. *Journal of Pharmacy and Pharmacology, 56*(12), 1527-1535.

Huang, C., and McIntosh, T. J. (1997). Probing the ethanol-induced chain interdigitations in gel-state bilayers of mixed-chain phosphatidylcholines. *Biophysical Journal, 72*(6), 2702-2709.

Huang, Z. M., Chinen, M., Chang, P. J., Xie, T., Zhong, L., Demetriou, S., Patel, M. P., Scherzer, R., Sviderskaya, E. V., and Bennett, D. C. (2012). Targeting protein-trafficking pathways alters melanoma treatment sensitivity. *Proceedings of the National Academy of Sciences, 109*(2), 553-558.

Humphrey, W., Dalke, A., and Schulten, K. (1996). VMD: Visual molecular dynamics. *Journal of Molecular Graphics, 14*(1), 33-38.

Hunter, R. J. (1981). *Zeta potential in colloid science: Principles and applications* (Vol. 125): Academic press London.

Hunter, R. J. (2001). Foundations of colloid science.

Huriez, C., and Thomas, P. (1977). Pevaryl in dermatology (apropos of 30 cases). *Lille Médical: Journal de la Faculté de Médecine et de Pharmacie de l'Université de Lille, 22*(4 Suppl 1), 272.

Hwang, H. Y., Kim, I. S., Kwon, I. C., and Kim, Y. H. (2008). Tumour targetability and antitumour effect of docetaxel-loaded hydrophobically modified glycol chitosan nanoparticles. *Journal of Controlled Release, 128*(1), 23-31.

Ibrahim, N. K., Desai, N., Legha, S., Soon-Shiong, P., Theriault, R. L., Rivera, E., Esmaeli, B., Ring, S. E., Bedikian, A., and Hortobagyi, G. N. (2002). Phase I and pharmacokinetic study of ABI-007, a Cremophor-free, protein-stabilised, nanoparticle formulation of paclitaxel. *Clinical Cancer Research, 8*(5), 1038-1044.

Ingólfsson, H. I., Koeppe Ii, R. E., and Andersen, O. S. (2011). Effects of green tea catechins on gramicidin channel function and inferred changes in bilayer properties. *FEBS Letters, 585*(19), 3101-3105.

Institute, N. C. (2010). *Cancer Nanotechnology Plan*.

Ito, T., Sun, L., Bevan, M. A., and Crooks, R. M. (2004). Comparison of nanoparticle size and electrophoretic mobility measurements using a carbon-nanotube-based coulter counter, dynamic light scattering, transmission electron microscopy, and phase analysis light scattering. *Langmuir, 20*(16), 6940-6945.

Jaiswal, J., Gupta, K. S., and Kreuter, J. (2004). Preparation of biodegradable cyclosporine nanoparticles by high pressure emulsification-solvent evaporation process. *Journal of Controlled Release, 96*(1), 169-178.

Jamil, H., Sheikh, S., and Ahmad, I. (2004). Liposomes. *Modern Drug Discovery*.

Jemal, A., Siegel, R., Xu, J., and Ward, E. (2010). Cancer statistics, 2010. *CA: A Cancer Journal for Clinicians, 60*(5), 277-300.

Jenning, V., Thünemann, A. F., and Gohla, S. H. (2000). Characterisation of a novel solid lipid nanoparticle carrier system based on binary mixtures of liquid and solid lipids. *International Journal of Pharmaceutics, 199*(2), 167-177.

Jeong, S. H., and Park, K. (2008). Drug loading and release properties of ion-exchange resin complexes as a drug delivery matrix. *International Journal of Pharmaceutics, 361*(1), 26-32.

Jordan, V. C. (2003). Tamoxifen: A most unlikely pioneering medicine. *Nature Reviews Drug Discovery, 2*(3), 205-213.

Jores, K., Mehnert, W., Drechsler, M., Bunjes, H., Johann, C., and Mäder, K. (2004). Investigations on the structure of solid lipid nanoparticles (SLN) and oil-loaded solid lipid nanoparticles by photon correlation spectroscopy, field-flow fractionation and transmission electron microscopy. *Journal of Controlled Release, 95*(2), 217-227.

Joshi, M., Pathak, S., Sharma, S., and Patravale, V. (2008). Design and *in vivo* pharmacodynamic evaluation of nanostructured lipid carriers for parenteral delivery of artemether: Nanoject. *International Journal of Pharmaceutics, 364*(1), 119-126.

Junghanns, J. U. A. H., and Müller, R. H. (2008). Nanocrystal technology, drug delivery and clinical applications. *International Journal of Nanomedicine, 3*(3), 295.

Kalam, M. A., Sultana, Y., Ali, A., Aqil, M., Mishra, A. K., and Chuttani, K. (2010). Preparation, characterisation, and evaluation of gatifloxacin loaded solid lipid nanoparticles as colloidal ocular drug delivery system. *Journal of Drug Targeting, 18*(3), 191-204.

Kanama, D. (2006). Patent application trends in the field of nanotechnology. *Quarterly Review, 21*, 77-88.

Kaszuba, M., McKnight, D., Connah, M. T., McNeil-Watson, F. K., and Nobbmann, U. (2008). Measuring sub nanometre sizes using dynamic light scattering. *Journal of Nanoparticle Research, 10*(5), 823-829.

Keenan, L., and Chapin, K. O. (2009). Laser Doppler Velocimetry.

Kelemen, K., Kiesecker, C., Zitron, E., Bauer, A., Scholz, E., Bloehs, R., Thomas, D., Greten, J., Remppis, A., and Schoels, W. (2007). Green tea flavonoid epigallocatechin-3-gallate (EGCG) inhibits cardiac hERG potassium channels. *Biochemical and Biophysical Research Communications, 364*(3), 429-435.

Khan, M. S., and Gowda, D. (2011). Nanoparticles: A boon for modernisation of drug delivery: A review. *International Journal of Nanoparticles, 4*(4), 389-411.

Kiaris, H., Chatzistamou, I., Kalofoutis, C., Koutselini, H., Piperi, C., and Kalofoutis, A. (2004). Tumour-stroma interactions in carcinogenesis: Basic aspects and perspectives. *Molecular and Cellular Biochemistry, 261*(1), 117-122.

Kim, J. T., Mattai, J., and Shipley, G. G. (1987). Bilayer interactions of ether- and ester-linked phospholipids: Dihexadecyl and dipalmitoylphosphatidylcholines. *Biochemistry, 26*(21), 6599-6603.

Kim, W. Y., Choi, Y. C., Min, S. K., Cho, Y., and Kim, K. S. (2009). Application of quantum chemistry to nanotechnology: Electron and spin transport in molecular devices. *Chemical Society Reviews, 38*(8), 2319-2333.

Kinnunen, P., Alakoskela, J. M., and Laggner, P. (2003). Phase behaviour of liposomes. *Methods in Enzymology, 367,* 129-147.

Kiwiel, K. C., and Murty, K. (1996). Convergence of the steepest descent method for minimizing quasiconvex functions. *Journal of Optimisation Theory and Applications, 89*(1), 221-226.

Koo, O. M., Rubinstein, I., and Onyuksel, H. (2005). Role of nanotechnology in targeted drug delivery and imaging: A concise review. *Nanomedicine: Nanotechnology, Biology and Medicine, 1*(3), 193-212.

Kranenburg, M., Vlaar, M., and Smit, B. (2004). Simulating induced interdigitation in membranes. *Biophysical Journal, 87*(3), 1596-1605.

Kumari, A., Yadav, S. K., and Yadav, S. C. (2010). Biodegradable polymeric nanoparticles based drug delivery systems. *Colloids and Surfaces B: Biointerfaces, 75*(1), 1-18.

Kuntsche, J., Westesen, K., Drechsler, M., Koch, M. H. J., and Bunjes, H. (2004). Supercooled smectic nanoparticles: A potential novel carrier system for poorly water soluble drugs. *Pharmaceutical Research, 21*(10), 1834-1843.

Lacatusu, I., Badea, N., Murariu, A., and Meghea, A. (2011). The encapsulation effect of UV molecular absorbers into biocompatible lipid nanoparticles. *Nanoscale Research Letters, 6*(1), 73.

Lamprecht, A., Ubrich, N., Hombreiro Perez, M., Lehr, C. M., Hoffman, M., and Maincent, P. (2000). Influences of process parameters on nanoparticle preparation performed by a double emulsion pressure homogenisation technique. *International Journal of Pharmaceutics, 196*(2), 177-182.

Lander, R., Manger, W., Scouloudis, M., Ku, A., Davis, C., and Lee, A. (2000). Gaulin homogenisation: A mechanistic study. *Biotechnology Progress, 16*(1), 80-85.

Lankalapalli, S., Routhu, K. C., Ojha, S., and Tenneti, V. S. V. K. (2014). Nanoparticulate drug delivery systems: Promissing approaches for drug delivery. *Journal of Drug Delivery and Therapeutics, special issue 1,* 72-85.

Lee, R. J. (2006). Liposomal delivery as a mechanism to enhance synergism between anticancer drugs. *Molecular Cancer Therapeutics, 5*(7), 1639-1640.

Leo, E., Brina, B., Forni, F., and Vandelli, M. A. (2004). In vitro evaluation of PLA nanoparticles containing a lipophilic drug in water-soluble or insoluble form. *International Journal of Pharmaceutics, 278*(1), 133-141.

Lewis, R. N. A. H., and McElhaney, R. N. (2007). Fourier-transform infrared spectroscopy in the study of lipid phase transitions in model and biological membranes. *Methods in Membrane Lipids, 400,* 207-226.

Li, C., Liu, H., Sun, Y., Wang, H., Guo, F., Rao, S., Deng, J., Zhang, Y., Miao, Y., and Guo, C. (2009). PAMAM nanoparticles promote acute lung injury by inducing autophagic cell death through the Akt-TSC2-mTOR signaling pathway. *Journal of Molecular Cell Biology, 1*(1), 37-45.

Li, S., Hattori, T., and Kodama, E. N. (2011). Epigallocatechin gallate inhibits the HIV reverse transcription step. *Antiviral Chemistry and Chemotherapy, 21*, 239-243.

Liu, C., Li, B. Q., and Mi, C. C. (2009). Fast transient thermal analysis of gold nanoparticles in tissue-like medium. *NanoBioscience, IEEE Transactions on, 8*(3), 271-280.

Liz-Marzán, L. M. (2005). Nanometals. *Reactions, 17*, 18.

Lohner, K., Latal, A., Degovics, G., and Garidel, P. (2001). Packing characteristics of a model system mimicking cytoplasmic bacterial membranes. *Chemistry and Physics of Lipids, 111*(2), 177-192.

Luchini, A., Fredolini, C., Espina, B. H., Meani, F., Reeder, A., Rucker, S., Petricoin Iii, E. F., and Liotta, L. A. (2010). Nanoparticle technology: Addressing the fundamental roadblocks to protein biomarker discovery. *Current Molecular Medicine, 10*(2), 133.

Lucks, S., and Müller, R. (1999). Medication vehicles made of solid lipid particles (solid lipid nanospheres-SLN): EP Patent 0,605,497.

Lundqvist, M., Stigler, J., Elia, G., Lynch, I., Cedervall, T., and Dawson, K. A. (2008). Nanoparticle size and surface properties determine the protein corona with possible implications for biological impacts. *Proceedings of the National Academy of Sciences, 105*(38), 14265-14270.

Luo, Y., Chen, D., Ren, L., Zhao, X., and Qin, J. (2006). Solid lipid nanoparticles for enhancing vinpocetine's oral bioavailability. *Journal of Controlled Release, 114*(1), 53-59.

Luzzati, V., and Chapman, D. (1968). Biological membranes. *Academic Press, New York*, 71-123.

Mabe, K., Yamada, M., Oguni, I., and Takahashi, T. (1999). *In vitro* and *in vivo* activities of tea catechins against *Helicobacter pylori*. *Antimicrobial Agents and Chemotherapy, 43*(7), 1788-1791.

Maeda, H., Bharate, G. Y., and Daruwalla, J. (2009). Polymeric drugs for efficient tumour-targeted drug delivery based on EPR-effect. *European Journal of Pharmaceutics and Biopharmaceutics, 71*(3), 409-419.

Malam, Y., Loizidou, M., and Seifalian, A. M. (2009). Liposomes and nanoparticles: Nanosized vehicles for drug delivery in cancer. *Trends in Pharmacological Sciences, 30*(11), 592-599.

Manthorpe, R., Petersen, S. H., and Prause, J. (1984). Mucosolvan in the treatment of patients with primary Sjögren's syndrome. *Acta Ophthalmologica, 62*(4), 537-541.

Martins, S., Sarmento, B., Ferreira, D. C., and Souto, E. B. (2007). Lipid-based colloidal carriers for peptide and protein delivery: Liposomes versus lipid nanoparticles. *International Journal of Nanomedicine, 2*(4), 595.

Mathur, V., Satrawala, Y., Rajput, M. S., Kumar, P., Shrivastava, P., and Vishvkarma, A. (2011). Solid lipid nanoparticles in cancer therapy. *International Journal of Drug Delivery, 2*(3).

Matsumura, Y., and Maeda, H. (1986). A new concept for macromolecular therapeutics in cancer chemotherapy: Mechanism of tumouritropic accumulation of proteins and the antitumour agent smancs. *Cancer Research, 46*(12 Part 1), 6387.

Maurelli, S., Chiesa, M., Giamello, E., Di Nardo, G., Ferrero, V. E. V., Gilardi, G., and Van Doorslaer, S. (2011). Direct spectroscopic evidence for binding of anastrozole to the iron heme of human aromatase. Peering into the mechanism of aromatase inhibition. *Chemical Communications, 47*(38), 10737-10739.

Mayer, C. (2002). Nuclear magnetic resonance on dispersed nanoparticles. *Progress in Nuclear Magnetic Resonance Spectroscopy, 40*(4), 307-366.

McClements, D. J. (2012). Nanoemulsions versus microemulsions: Terminology, differences, and similarities. *Soft Matter, 8*(6), 1719-1729.

McGill, S. L., Cuylear, C. L., Adolphi, N. L., Osinski, M., and Smyth, H. D. C. (2009). Magnetically responsive nanoparticles for drug delivery applications using low magnetic field strengths. *NanoBioscience, IEEE Transactions on, 8*(1), 33-42.

Mehnert, W., and Mäder, K. (2001). Solid lipid nanoparticles: Production, characterisation and applications. *Advanced Drug Delivery Reviews, 47*(2-3), 165-196.

Mehnert, W., and Mäder, K. (2012). Solid lipid nanoparticles production, characterisation and applications. *Advanced Drug Delivery Reviews*.

Meinzer, A., Müller, E., and Vonderscher, J. (1998). Perorale microemulsionsformulierung-Sandimmun Optoral®/Neoral®. In R. H. Müller and G. E. Hildebrand (Eds.), *Pharmazeutische Technologie: Moderne Arzneiformen* (pp. 169-177). Stuttgart: Wissenschaftliche Verlagsgesellschaft GmbH.

Meißner, T., Potthoff, A., and Richter, V. (2009). *Suspension characterisation as important key for toxicological investigations.* Paper presented at the Journal of Physics: Conference Series.

Merisko-Liversidge, E., Liversidge, G. G., and Cooper, E. R. (2003). Nanosizing: A formulation approach for poorly-water-soluble compounds. *European Journal of Pharmaceutical Sciences, 18*(2), 113-120.

Miyamoto, S., and Kollman, P. A. (1992). SETTLE: An analytical version of the SHAKE and RATTLE algorithm for rigid water models. *Journal of Computational Chemistry, 13*(8), 952-962.

Mokrushin, S. G. (1962). Thomas Graham and the Definition of Colloids. *Nature,* 861.

Montes-Burgos, I., Walczyk, D., Hole, P., Smith, J., Lynch, I., and Dawson, K. (2010). Characterisation of nanoparticle size and state prior to nanotoxicological studies. *Journal of Nanoparticle Research, 12*(1), 47-53.

Mouridsen, H., Gershanovich, M., Sun, Y., Pérez-Carrión, R., Boni, C., Monnier, A., Apffelstaedt, J., Smith, R., Sleeboom, H. P., and Jaenicke, F. (2003). Phase III study of letrozole versus tamoxifen as first-line therapy of advanced breast cancer in postmenopausal women: Analysis of survival and update of efficacy from the International Letrozole Breast Cancer Group. *Journal of Clinical Oncology, 21*(11), 2101-2109.

Muchow, M., Maincent, P., and Müller, R. H. (2008). Lipid nanoparticles with a solid matrix (SLN®, NLC®, LDC®) for oral drug delivery. *Drug Development and Industrial Pharmacy, 34*(12), 1394-1405.

Mukhtar, H., and Ahmad, N. (2000). Tea polyphenols: Prevention of cancer and optimizing health. *The American Journal of Clinical Nutrition, 71*(6), 1698-1702.

Müller, R. H., and Lucks, J. S. (1996). European Patent No. EP0605497.

Müller, R. H., Mäder, K., and Gohla, S. (2000). Solid lipid nanoparticles (SLN) for controlled drug delivery: a review of the state of the art. *European Journal of Pharmaceutics and Biopharmaceutics, 50*(1), 161-177.

Müller, R. H., Moschwitzer, J., and Bushrab, F. N. (2006). Manufacturing of nanoparticles by milling and homogenisation techniques. *Drugs and the pharmaceutical sciences, 159*, 21.

Müller, R. H., Radtke, M., and Wissing, S. A. (2002a). Nanostructured lipid matrices for improved microencapsulation of drugs. *International Journal of Pharmaceutics, 242*(1), 121-128.

Müller, R. H., Radtke, M., and Wissing, S. A. (2002b). Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations. *Advanced Drug Delivery Reviews, 54*, S131-S155.

Müller, R. H., Radtke, M., and Wissing, S. A. (2004). Solid lipid nanoparticles and nanostructured lipid carriers. In H. S. Nalwa (Ed.), *Encyclopedia of Nanoscience and Nanotechnology*. Los Angeles, California: Encyclopedia of Nanoscience and Nanotechnology.

Müller, R. H., Rühl, D., Runge, S., Schulze-Forster, K., and Mehnert, W. (1997). Cytotoxicity of solid lipid nanoparticles as a function of the lipid matrix and the surfactant. *Pharmaceutical Research, 14*(4), 458-462.

Myhre, S., Mohammed, H., Tramm, T., Alsner, J., Finak, G., Park, M., Overgaard, J., Børresen-Dale, A. L., Frigessi, A., and Sørlie, T. (2010). In silico ascription of gene expression differences to tumour and stromal cells in a model to study impact on breast cancer outcome. *PLoS One, 5*(11), e14002.

Nagle, D. G., Ferreira, D., and Zhou, Y. D. (2006). Epigallocatechin-3-gallate (EGCG): Chemical and biomedical perspectives. *Phytochemistry, 67*(17), 1849-1855.

Nakayama, T., Hashimoto, T., Kajiya, K., and Kumazawa, S. (2000). Affinity of polyphenols for lipid bilayers. *Biofactors, 13*(1), 147-151.

Nanda, K. K., Maisels, A., Kruis, F. E., Fissan, H., and Stappert, S. (2003). Higher surface energy of free nanoparticles. *Physical Review Letters, 91*(10), 106102.

Näreoja, T., Vehniäinen, M., Lamminmäki, U., Hänninen, P. E., and Härmä, H. (2009). Study on nonspecificity of an immuoassay using Eu-doped polystyrene nanoparticle labels. *Journal of Immunological Methods, 345*(1), 80-89.

National Institute of Environmental Health Sciences (2001). Report of the international workshop on *in vitro* methods for assessing acute systemic toxicity. *NIH Publication*(01-4499).

Navarro-Perán, E., Cabezas-Herrera, J., Sánchez-del-Campo, L., and Rodríguez-López, J. N. (2007). Effects of folate cycle disruption by the green tea polyphenol epigallocatechin-3-gallate. *The International Journal of Biochemistry and Cell Biology, 39*(12), 2215-2225.

Navarro-Perán, E., Cabezas-Herrera, J., García-Cánovas, F., Durrant, M. C., Thorneley, R. N., and Rodríguez-López, J. N. (2005). The antifolate activity of tea catechins. *Cancer Research, 65*(6), 2059-2064.

O'brien, M., Wigler, N., Inbar, M., Rosso, R., Grischke, E., Santoro, A., Catane, R., Kieback, D., Tomczak, P., and Ackland, S. (2004). Reduced cardiotoxicity and comparable efficacy in a phase III trial of pegylated liposomal doxorubicin HCl (CAELYX™/Doxil®) versus conventional doxorubicin for first-line treatment of metastatic breast cancer. *Annals of Oncology, 15*(3), 440-449.

Oberdörster, G., Oberdörster, E., and Oberdörster, J. (2005). Nanotoxicology: An emerging discipline evolving from studies of ultrafine particles. *Environmental health perspectives, 113*(7), 823.

Olbrich, C., Kayser, O., and Müller, R. H. (2002). Lipase degradation of Dynasan 114 and 116 solid lipid nanoparticles (SLN): Effect of surfactants, storage time and crystallinity. *International Journal of Pharmaceutics, 237*(1), 119-128.

Olbrich, C., and Müller, R. H. (1999). Enzymatic degradation of SLN: Effect of surfactant and surfactant mixtures. *International Journal of Pharmaceutics, 180*(1), 31-39.

Ortiz, A., Teruel, J. A., Espuny, M. J., Marqués, A., Manresa, Á., and Aranda, F. J. (2008). Interactions of a *Rhodococcus sp*. biosurfactant trehalose lipid with phosphatidylethanolamine membranes. *Biochimica et Biophysica Acta (BBA)-Biomembranes, 1778*(12), 2806-2813.

Pabst, G. (2006). Global properties of biomimetic membranes: Perspectives on molecular features. *Biophysical Reviews and Letters, 1*(1), 57-84.

Pabst, G., Danner, S., Karmakar, S., Deutsch, G., and Raghunathan, V. A. (2007). On the propensity of phosphatidylglycerols to form interdigitated phases. *Biophysical Journal, 93*(2), 513-525.

Pabst, G., Koschuch, R., Pozo-Navas, B., Rappolt, M., Lohner, K., and Laggner, P. (2003). Structural analysis of weakly ordered membrane stacks. *Journal of Spplied Crystallography, 36*(6), 1378-1388.

Pabst, G., Rappolt, M., Amenitsch, H., and Laggner, P. (2000). Structural information from multilamellar liposomes at full hydration: Full q-range fitting with high quality X-ray data. *Physical Review E, 62*(3), 4000.

Panyam, J., Dali, M. M., Sahoo, S. K., Ma, W., Chakravarthi, S. S., Amidon, G. L., Levy, R. J., and Labhasetwar, V. (2003a). Polymer degradation and in vitro release of a model protein from poly(d,l-lactide-*co*-glycolide) nano-and microparticles. *Journal of Controlled Release, 92*(1), 173-187.

Panyam, J., Sahoo, S. K., Prabha, S., Bargar, T., and Labhasetwar, V. (2003b). Fluorescence and electron microscopy probes for cellular and tissue uptake of poly (d,l-lactide-*co*-glycolide) nanoparticles. *International Journal of Pharmaceutics, 262*(1), 1-11.

Park, B. J., Park, J. C., Taguchi, H., Fukushima, K., Hyon, S. H., and Takatori, K. (2006). Antifungal susceptibility of epigallocatechin 3-O-gallate (EGCg) on clinical isolates of pathogenic yeasts. *Biochemical and Biophysical Research Communications, 347*(2), 401-405.

Pathan, I. B., and Setty, C. M. (2011). Enhancement of transdermal delivery of tamoxifen citrate using nanoemulsion vehicle. *International Journal of PharmTech Research, 3*(1), 287-297.

Patra, S. K., Rizzi, F., Silva, A., Rugina, D. O., and Bettuzzi, S. (2008). Molecular targets of (–)-epigallocatechin-3-gallate (EGCG): Specificity and interaction with membrane lipid rafts. *Journal of Physiology and Pharmacology, 59*(Suppl 9), 217-235.

Pecora, R. (2000). Dynamic light scattering measurement of nanometer particles in liquids. *Journal of Nanoparticle Research, 2*(2), 123-131.

Pękal, A., Dróżdż, P., Biesaga, M., and Pyrzynska, K. (2012). Screening of the antioxidant properties and polyphenol composition of aromatised green tea infusions. *Journal of the Science of Food and Agriculture, 92*(11), 2244-2249.

Pérez-Lara, A., Ausili, A., Aranda, F. J., Godos, A. d., Torrecillas, A., Corbalán-García, S., and Gómez-Fernández, J. C. (2010). Curcumin Disorders 1, 2-dipalmitoyl-sn-glycero-3-phosphocholine Membranes and Favors the Formation of Nonlamellar Structures by 1,2-dielaidoyl-*sn*-glycero-3-phosphoethanolamine. *The Journal of Physical Chemistry B, 114*(30), 9778-9786.

Petri-Fink, A., and Hofmann, H. (2007). Superparamagnetic iron oxide nanoparticles (SPIONs): From synthesis to *in vivo* studies: A summary of the synthesis, characterisation, *in vitro*, and *in vivo* investigations of SPIONs with particular focus on surface and colloidal properties. *NanoBioscience, IEEE Transactions on, 6*(4), 289-297.

Pitkethly, M. J. (2004). Nanomaterials: The driving force. *Materials Today, 7*(12), 20-29.

Prankerd, R. J., and Stella, V. J. (1990). The use of oil-in-water emulsions as a vehicle for parenteral drug administration. *PDA Journal of Pharmaceutical Science and Technology, 44*(3), 139-149.

Pronk, S., Páll, S., Schulz, R., Larsson, P., Bjelkmar, P., Apostolov, R., Shirts, M. R., Smith, J. C., Kasson, P. M., and van der Spoel, D. (2013). GROMACS 4.5: A high-throughput and highly parallel open source molecular simulation toolkit. *Bioinformatics, 29*(7), 845-854.

Radomska-Soukharev, A. (2007). Stability of lipid excipients in solid lipid nanoparticles. *Advanced Drug Delivery Reviews, 59*(6), 411-418.

Repáková, J., Capková, P., Holopainen, J. M., and Vattulainen, I. (2004). Distribution, orientation, and dynamics of DPH probes in DPPC bilayer. *The Journal of Physical Chemistry B, 108*(35), 13438-13448.

Repáková, J., Holopainen, J. M., Morrow, M. R., McDonald, M. C., Čapková, P., and Vattulainen, I. (2005). Influence of DPH on the structure and dynamics of a DPPC bilayer. *Biophysical Journal, 88*(5), 3398-3410.

Robertson, J. D. (1966). Granulo-fibrillar and globular substructure in unit membranes. *Annals of the New York Academy of Sciences, 137*(2), 421-440.

Rodríguez-López, J. N., Sánchez-del-Campo, L., Sáez-Ayala, M., Montenegro, M. F., and Cabezas-Herrera, J. (2011). Novel Antifolates as Produgs for the Treatment of Melanoma. In M. Murph (Ed.), *Research on melanoma: A glimpse into current directions of future trends*. Rijeka: InTech.

Sáez-Ayala, M., Montenegro, M. F., Sánchez-del-Campo, L., Fernández-Pérez, M. P., Chazarra, S., Freter, R., Middleton, M., Piñero-Madrona, A., Cabezas-Herrera, J., and Goding, C. R. (2013). Directed phenotype switching as an effective antimelanoma strategy. *Cancer Cell, 24*(1), 105-119.

Sáez-Ayala, M., Sánchez-del-Campo, L., Montenegro, M. F., Chazarra, S., Tárraga, A., Cabezas-Herrera, J., and Rodríguez-López, J. N. (2011). Comparison of a Pair of Synthetic Tea-Catechin-Derived Epimers: Synthesis, Antifolate Activity, and Tyrosinase-Mediated Activation in Melanoma. *ChemMedChem, 6*(3), 440-449.

Samad, A., Sultana, Y., and Aqil, M. (2007). Liposomal drug delivery systems: An update review. *Current Drug Delivery, 4*(4), 297-305.

Sánchez-del-Campo, L., Otón, F., Tárraga, A., Cabezas-Herrera, J., Chazarra, S., and Rodríguez-López, J. N. (2008). Synthesis and biological activity of a 3,4,5-trimethoxybenzoyl ester analogue of epicatechin-3-gallate. *Journal of Medicinal Chemistry, 51*(7), 2018-2026.

Sánchez-del-Campo, L., Tárraga, A., Montenegro, M. F., Cabezas-Herrera, J., and Rodríguez-López, J. N. (2009). Melanoma activation of 3-O-(3,4,5-trimethoxybenzoyl)-(–)-epicatechin to a potent irreversible inhibitor of dihydrofolate reductase. *Molecular Pharmaceutics, 6*(3), 883-894.

Sánchez-del-Campo, L., Chazarra, S., Montenegro, M. F., Cabezas-Herrera, J., and Rodríguez-López, J. N. (2010). Mechanism of dihydrofolate reductase downregulation in melanoma by 3-O-(3,4,5-trimethoxybenzoyl)-(–)-epicatechin. *Journal of Cellular Biochemistry, 110*(6), 1399-1409.

Sánchez-del-Campo, L., Montenegro, M. F., Cabezas-Herrera, J., and Rodríguez-López, J. N. (2009). The critical role of alpha-folate receptor in the resistance of melanoma to methotrexate. *Pigment Cell and Melanoma Research, 22*(5), 588-600.

Sánchez-del-Campo, L., and Rodríguez-López, J. N. (2008). Targeting the methionine cycle for melanoma therapy with 3-O-(3,4,5-trimethoxybenzoyl)-(–)-epicatechin. *International Journal of Cancer, 123*(10), 2446-2455.

Santos, P., Watkinson, A. C., Hadgraft, J., and Lane, M. E. (2008). Application of microemulsions in dermal and transdermal drug delivery. *Skin Pharmacology and Physiology, 21*(5), 246-259.

Saunders, L., and Thomas, I. L. (1958). Diffusion studies with lysolecithin. *Journal of the Chemical Society*, *85*, 483-485.

Schäfer-Korting, M., Mehnert, W., and Korting, H. C. (2007). Lipid nanoparticles for improved topical application of drugs for skin diseases. *Advanced Drug Delivery Reviews, 59*(6), 427-443.

Schlupp, P., Blaschke, T., Kramer, K., Höltje, H. D., Mehnert, W., and Schäfer-Korting, M. (2011). Drug release and skin penetration from solid lipid nanoparticles and a base cream: A systematic approach from a comparison of three glucocorticoids. *Skin Pharmacology and Physiology, 24*(4), 199-209.

Schmidt, M. W., Baldridge, K. K., Boatz, J. A., Elbert, S. T., Gordon, M. S., Jensen, J. H., Koseki, S., Matsunaga, N., Nguyen, K. A., and Su, S. (1993). General atomic and molecular electronic structure system. *Journal of Computational Chemistry, 14*(11), 1347-1363.

Schmitt, J. (1998). Parenterale Fettemulsionen als Arzneistoffträger. In R. H. Müller and G. E. Hildebrand (Eds.), *Moderne Arzneiformen* (pp. 137-142). Stuttgar: Wissenschaftliche Verlagsgesellschaft.

Schöpe, H. J., Bryant, G., and van Megen, W. (2006). Small changes in particle-size distribution dramatically delay and enhance nucleation in hard sphere colloidal suspensions. *Physical Review E, 74*(6), 060401.

Schrödinger, L. L. C. (2010). The PyMOL molecular graphics system, version 1.3 r1. *The PyMOL Molecular Graphics System, Version, 1*.

Schubert, M. A., and Müller-Goymann, C. C. (2005). Characterisation of surface-modified solid lipid nanoparticles (SLN): Influence of lecithin and nonionic emulsifier. *European Journal of Pharmaceutics and Biopharmaceutics, 61*(1), 77-86.

Schubert, M. A., Schicke, B. C., and Müller-Goymann, C. C. (2005). Thermal analysis of the crystallisation and melting behavior of lipid matrices and lipid nanoparticles containing high amounts of lecithin. *International Journal of Pharmaceutics, 298*(1), 242-254.

Schuster, J., Rubsamen, R., Lloyd, P., and Lloyd, J. (1997). The AERx™ aerosol delivery system. *Pharmaceutical Research, 14*(3), 354-357.

Schuttelkopf, A. W., and Van Aalten, D. M. F. (2004). PRODRG: A tool for high-throughput crystallography of protein-ligand complexes. *Acta Crystallographica Section D: Biological Crystallography, 60*(8), 1355-1363.

Sernelius, B. E. (2001). Surface mode in physics. Berlin: Wiley-VCH.

Serpe, L., Catalano, M. G., Cavalli, R., Ugazio, E., Bosco, O., Canaparo, R., Muntoni, E., Frairia, R., Gasco, M. R., and Eandi, M. (2004). Cytotoxicity of anticancer drugs incorporated in solid lipid nanoparticles on HT-29 colorectal cancer cell line. *European Journal of Pharmaceutics and Biopharmaceutics, 58*(3), 673-680.

Shah, K. A., Joshi, M. D., and Patravale, V. B. (2009). Biocompatible microemulsions for fabrication of glyceryl monostearate solid lipid nanoparticles (SLN) of tretinoin. *Journal of Biomedical Nanotechnology, 5*(4), 396-400.

Sharma, J., Kalra, S., Sharma, A., and Rani, S. (2010). Colloidal drug carriers. *The Internet Journal of Family Practice, 9*(2), 17.

Shegokar, R., and Müller, R. H. (2010). Nanocrystals: Industrially feasible multifunctional formulation technology for poorly soluble actives. *International Journal of Pharmaceutics, 399*(1-2), 129-139.

Shi, X., Ye, J., Leonard, S., Ding, M., Vallyathan, V., Castranova, V., Rojanasakul, Y., and Dong, Z. (2000). Antioxidant properties of (–)-epicatechin-3-gallate and its inhibition of Cr (VI)-induced DNA damage and Cr (IV)-or TPA-stimulated NF-κB activation. *Molecular and Cellular Biochemistry, 206*(1-2), 125-132.

Siegel, R., Ward, E., Brawley, O., and Jemal, A. (2011). Cancer statistics, 2011. *CA: A Cancer Journal for Clinicians, 61*(4), 212-236.

Silva, H. D., Cerqueira, M. A., Souza, B. W. S., Ribeiro, C., Avides, M. C., Quintas, M. A. C., Coimbra, J. S. R., Carneiro-da-Cunha, M. G., and Vicente, A. A. (2011). Nanoemulsions of β-carotene using a high-energy emulsification-evaporation technique. *Journal of Food Engineering, 102*(2), 130-135.

Singh, M., and O'Hagan, D. (1998). The preparation and characterisation of polymeric antigen delivery systems for oral administration. *Advanced Drug Delivery Reviews, 34*(2-3), 285-304.

Sinha, V. R., Bansal, K., Kaushik, R., Kumria, R., and Trehan, A. (2004). Poly-ϵ-caprolactone microspheres and nanospheres: An overview. *International Journal of Pharmaceutics, 278*(1), 1-23.

Song, J. M., Lee, K. H., and Seong, B. L. (2005). Antiviral effect of catechins in green tea on influenza virus. *Antiviral Research, 68*(2), 66-74.

Soppimath, K. S., Aminabhavi, T. M., Kulkarni, A. R., and Rudzinski, W. E. (2001). Biodegradable polymeric nanoparticles as drug delivery devices. *Journal of Controlled Release, 70*(1), 1-20.

Souto, E. B., and Müller, R. H. (2005). SLN and NLC for topical delivery of ketoconazole. *Journal of Microencapsulation, 22*(5), 501-510.

Souto, E. B., and Müller, R. H. (2006). Investigation of the factors influencing the incorporation of clotrimazole in SLN and NLC prepared by hot high-pressure homogenisation. *Journal of Microencapsulation, 23*(4), 377-388.

Souto, E. B., and Müller, R. H. (2007). Lipid nanoparticles (solid lipid nanoparticles and nanostructured lipid carriers) for cosmetic, dermal, and transdermal applications. In D. Thassu, M. Deleers and Y. Pathak (Eds.), *Drugs and the Pharmaceutical Science: Nanoparticulate Drug Delivery Systems* (pp. 213-234). North Carolina: PharmaceuTech Inc.

Souto, E. B., and Müller, R. H. (2008). Cosmetic features and applications of lipid nanoparticles (SLN®, NLC®). *International Journal of Cosmetic Science, 30*(3), 157-165.

Souto, E. B., Wissing, S. A., Barbosa, C. M., and Müller, R. H. (2004). Development of a controlled release formulation based on SLN and NLC for topical clotrimazole delivery. *International Journal of Pharmaceutics, 278*(1), 71-77.

Speiser, P. (1990). European Patent No. EP0167825.

Steinmann, J., Buer, J., Pietschmann, T., and Steinmann, E. (2013). Anti-infective properties of epigallocatechin-3-gallate (EGCG), a component of green tea. *British Journal of Pharmacology, 168*(5), 1059-1073.

Sun, Y., Hung, W. C., Chen, F. Y., Lee, C. C., and Huang, H. W. (2009). Interaction of tea catechin (–)-epigallocatechin gallate with lipid bilayers. *Biophysical Journal, 96*(3), 1026-1035.

Tadros, T. F. (2006). *Applied surfactants: Principles and applications*. Weinheim: John Wiley and Sons.

Takagi, T. (2005). Electrophoretic light scattering. *Electrophoresis, 14*(1), 1255-1256.

Tamba, Y., Ohba, S., Kubota, M., Yoshioka, H., Yoshioka, H., and Yamazaki, M. (2007). Single GUV method reveals interaction of tea catechin (–)-epigallocatechin gallate with lipid membranes. *Biophysical Journal, 92*(9), 3178-3194.

Tardieu, A., Luzzati, V., and Reman, F. C. (1973). Structure and polymorphism of the hydrocarbon chains of lipids: A study of lecithin-water phases. *Journal of Molecular Biology, 75*(4), 711-733.

Taroco, H. A., Santos, J. A. F., Domingues, R. Z., and Matencio, T. (2011). Ceramic Materials for Solid Oxide Fuel Cells.

Teruel, J. A., Ortiz, A., and Aranda, F. J. (2004). Influence of organotin compounds on phosphatidylserine membranes. *Applied Organometallic Chemistry, 18*(3), 111-116.

Tiwari, R., and Pathak, K. (2011). Nanostructured lipid carrier versus solid lipid nanoparticles of simvastatin: Comparative analysis of characteristics, pharmacokinetics and tissue uptake. *International Journal of Pharmaceutics, 415*(1), 232-243.

Torchilin, V. (2011). Tumour delivery of macromolecular drugs based on the EPR effect. *Advanced Drug Delivery Reviews, 63*(3), 131-135.

Torchilin, V. P. (2006). *Nanoparticulates as drug carriers*: Imperial college press.

Touitou, E., Junginger, H. E., Weiner, N. D., Nagai, T., and Mezei, M. (1994). Liposomes as carriers for topical and transdermal delivery. *Journal of Pharmaceutical Sciences, 83*(9), 1189-1203.

Trost, N., Juvan, P., Sersa, G., and Debeljak, N. (2012). Contrasting effect of recombinant human erythropoietin on breast cancer cell response to cisplatin induced cytotoxicity. *Radiology and Oncology, 46*(3), 213-225.

Uekusa, Y., Kamihira, M., and Nakayama, T. (2007). Dynamic behavior of tea catechins interacting with lipid membranes as determined by NMR spectroscopy. *Journal of Agricultural and Food Chemistry, 55*(24), 9986-9992.

Ulrich, A. S., Volke, F., and Watts, A. (1990). The dependence of phospholipid head-group mobility on hydration as studied by deuterium-NMR spin-lattice relaxation time measurements. *Chemistry and Physics of Lipids, 55*(1), 61-66.

van Gunsteren, W. F., Billeter, S. R., Eising, A. A., Hünenberger, P. H., Krüger, P., Mark, A. E., Scott, W. R. P., and Tironi, I. G. (1996). Biomolecular Simulation: The GROMOS96 manual and user guide.

Varshosaz, J., Eskandari, S., Kennedy, R., Tabbakhian, M., and Minaiyan, M. (2013). Factors affecting the production of nanostructure lipid carriers of valproic acid. *Journal of Biomedical Nanotechnology, 9*(2), 202-212.

Vyas, T. K., Shahiwala, A., and Amiji, M. M. (2008). Improved oral bioavailability and brain transport of Saquinavir upon administration in novel nanoemulsion formulations. *International Journal of Pharmaceutics, 347*(1), 93-101.

Wagner, V., Dullaart, A., Bock, A. K., and Zweck, A. (2006). The emerging nanomedicine landscape. *Nature Biotechnology, 24*(10), 1211-1218.

Wallace, W. E., Keane, M. J., Murray, D. K., Chisholm, W. P., Maynard, A. D., and Ong, T. (2007). Phospholipid lung surfactant and nanoparticle surface toxicity: Lessons from diesel soots and silicate dusts. *Nanotechnology and Occupational Health*, 23-38.

Wan, F., You, J., Sun, Y., Zhang, X. G., Cui, F. D., Du, Y. Z., Yuan, H., and Hu, F.-Q. (2008). Studies on PEG-modified SLNs loading vinorelbine bitartrate (I): Preparation and evaluation *in vitro*. *International Journal of Pharmaceutics, 359*(1), 104-110.

Wang, R., Li, L., Wang, B., Zhang, T., and Sun, L. (2012). FK506-loaded solid lipid nanoparticles: Preparation, characterisation and *in vitro* transdermal drug delivery. *African Journal of Pharmaceutics and Pharmacology, 6*(12), 904-913.

Weissman, G., Sessa, G., Standish, M., and Bangham, A. D. (1965). A direct effect of steroids on permeability of lipid membranes (liposomes). *Journal of Clinical Investigation, 44*(6), 1109.

Westesen, K., Bunjes, H., and Koch, M. H. J. (1997). Physicochemical characterisation of lipid nanoparticles and evaluation of their drug loading capacity and sustained release potential. *Journal of Controlled Release, 48*(2), 223-236.

Westesen, K., and Siekmann, B. (1997). Investigation of the gel formation of phospholipid-stabilised solid lipid nanoparticles. *International Journal of Pharmaceutics, 151*(1), 35-45.

Wong-Ekkabut, J., Miettinen, M. S., Dias, C., and Karttunen, M. (2010). Static charges cannot drive a continuous flow of water molecules through a carbon nanotube. *Nature Nanotechnology, 5*(8), 555-557.

Wood, M. E., Fama, T. A., Ashikaga, T., and Muss, H. B. (2010). Discrepancy between rreference and actual adjuvant therapy for breast cancer. *Clinical Breast Cancer, 10*(5), 398-403.

Xie, T., Nguyen, T., Hupe, M., and Wei, M. L. (2009). Multidrug resistance decreases with mutations of melanosomal regulatory genes. *Cancer Research, 69*(3), 992-999.

Yang, C. S., Lambert, J. D., Ju, J., Lu, G., and Sang, S. (2007). Tea and cancer prevention: Molecular mechanisms and human relevance. *Toxicology and Applied Pharmacology, 224*(3), 265-273.

Yokozawa, T., Cho, E. J., Hara, Y., and Kitani, K. (2000). Antioxidative activity of green tea treated with radical initiator 2, 2'-azobis (2-amidinopropane) dihydrochloride. *Journal of Agricultural and Food Chemistry, 48*(10), 5068-5073.

Yoshimatsu, K., Reimhult, K., Krozer, A., Mosbach, K., Sode, K., and Ye, L. (2007). Uniform molecularly imprinted microspheres and nanoparticles prepared by precipitation polymerisation: The control of particle size suitable for different analytical applications. *Analytica Chimica Acta, 584*(1), 112-121.

Yuan, Y., Gao, Y., Zhao, J., and Mao, L. (2008). Characterisation and stability evaluation of β-carotene nanoemulsions prepared by high pressure homogenisation under various emulsifying conditions. *Food Research International, 41*(1), 61-68.

Zauner, W., Farrow, N. A., and Haines, A. M. R. (2001). *In vitro* uptake of polystyrene microspheres: Effect of particle size, cell line and cell density. *Journal of Controlled Release, 71*(1), 39-51.

Zhang, Y. P., Lewis, R. N. A. H., and McElhaney, R. N. (1997). Calorimetric and Spectroscopic Studies of the Thermotropic Phase Behavior of the *n*-Saturated 1,2-diacylphosphatidylglycerols. *Biophysical Journal, 72*(2), 779-793.

Zheng, J., and Ramirez, V. D. (2000). Inhibition of mitochondrial proton F0F1-ATPase/ATP synthase by polyphenolic phytochemicals. *British Journal of Pharmacology, 130*(5), 1115-1123.

Zhu, C. Q., Popova, S. N., Brown, E. R. S., Barsyte-Lovejoy, D., Navab, R., Shih, W., Li, M., Lu, M., Jurisica, I., and Penn, L. Z. (2007). Integrin α11 regulates IGF2 expression in fibroblasts to enhance tumourigenicity of human non-small-cell lung cancer cells. *Proceedings of the National Academy of Sciences, 104*(28), 11754-11759.

Zhuang, C. Y., Li, N., Wang, M., Zhang, X. N., Pan, W. S., Peng, J. J., Pan, Y. S., and Tang, X. (2010). Preparation and characterisation of vinpocetine loaded nanostructured lipid carriers (NLC) for improved oral bioavailability. *International Journal of Pharmaceutics, 394*(1), 179-185.

Zimmermann, E., Souto, E. B., and Muller, R. H. (2005). Physicochemical investigations on the structure of drug-free and drug-loaded solid lipid nanoparticles (SLNTM) by means of DSC and 1H NMR. *Die Pharmazie - An International Journal of Pharmaceutical Sciences, 60*(7), 508-513.

zur Mühlen, A., Schwarz, C., and Mehnert, W. (1998). Solid lipid nanoparticles (SLN) for controlled drug delivery-drug release and release mechanism. *European Journal of Pharmaceutics and Biopharmaceutics, 45*(2), 149-155.