

Drug carriers in cancer therapy: Administration, formulation and characterization

Rusnah Syahila Duali Hussen* and Thorsten Heidelberg

Chemistry Department, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia.

ABSTRACT

Besides the active ingredient, matters of administration are of tremendous importance for the performance of a drug. A variety of carriers, differing in morphology and composition can be applied to enhance the efficiency of pharmaceutical active compounds. This review addresses common administration routes for cancer chemotherapy, i.e. intravenous injection, oral and transdermal application, and presents related carriers. Emphasis has been placed on vesicular systems, which are particularly useful for intravenous and oral administration. In view of considerable morphological impacts on the performance of a drug, methods for the physico-chemical characterization of carriers, covering size, encapsulation efficiency and drug release, are addressed as well.

Keywords: Administration of anticancer drugs, drug delivery, nanotubes, nanoparticles, vesicles

Received 1 Feb 2016

Received in revised form 27 Apr 2016

Accepted 30 Apr 2016

*Address for correspondence

Rusnah S.D. Hussen,

Chemistry Department, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia.

E-mail: r_syahila@um.edu.my

INTRODUCTION

Cancer is a disease caused by uncontrolled growth of cells inside an organism. According to the World Cancer Report 2014, cancer is the leading cause of death worldwide. In many case the tumour, i.e. the core aggregation of cancer cells, can be removed by surgery. However, in order to remove metastases, referring to spread new colonies of cancerous cells, an additional therapy with anti-cancer drugs, known as chemotherapy, is frequently required. Many anticancer drugs target the DNA replication, since the uncontrolled division of cells is the source of disease [1,2]. Anticancer drugs can block the nucleic acid biosynthesis, damage the structure and function of DNA, interfere transportation or block the RNA or subsequent protein biosynthesis of key features for the mitosis [3]. Less common approaches aim on the interactions of cancerous cells, e.g. hormones and the supply of nutrients [4]. The typical interaction target mitosis requires cancer drugs to enter the cell prior to their medical action.

The chemical nature of anticancer drugs is divers, covering both hydrophilic [5,6] and hydrophobic active compounds [7]. A typical problem of chemotherapy is the non-cell-specific action of the drugs, which commonly affect any cell at the stage of division. This leads to severe side effects, which is Hussen RSD et al. IJPRR2016;5(4)

visualized in the loss of hair for chemotherapy patients [8]. In order reduce the toxic side effects, localized application of drugs is important. For this the application of a delivery system is crucial. Besides restriction of drug distribution to the targeted location a delivery system should address the transfer of the cancer drug into the target cell as well.

ADMINISTRATION OF ANTICANCER DRUGS

Intravenous administration

The intravenous administration is a method, in which the medication is injected into a vein through a needle or tube. This method is the most commonly used to achieve either immediate accessibility of the drug in the bloodstream, e.g. for emergency treatments, or to avoid its damage on the way through the gastrointestinal (GI) tract owing to acid- or enzyme-induced reactions. Suitable anticancer drug carriers for intravenous injection administration involve nanotubes [9], nanoparticles, [10] vesicles [10] and emulsions [11]. While this administration type provides the lowest constraints in terms of compound compatibility, patients typically dislike the injections. Besides, the latter can cause medical problems due to extravasation of drug or blood, catheter infections, and thrombosis [12].

Oral administration

Oral intake of medication requires absorption through the gastrointestinal (GI) tract. The main advantage of this administration is convenience for the patients, which is particularly important for paediatric patients, including elderly who need to take medications regularly and continuously. Oral medication can prevent the pain and discomfort associated with injections and avoids the possibility of infections caused by inappropriate use or reuse of needles. Potential biodegradation inside the GI system limits oral applications of delicate drugs unless a suitable drug carrier offers protection. Typical carriers for oral drug administration cover emulsions, [13] microspheres, [14] liposomes [15] and nanoparticles [16].

Transdermal administration

In transdermal administration the active ingredients are placed on the surface of the skin to be absorbed into the systemic circulation. This method is not widely used, because the skin typically exhibits low permeability for compounds. However, if this problem can be overcome, transdermal drug delivery provides advantages over both oral and intravenous administration. Most eminent are patients acceptance on the one hand, and bypassing the challenging gastrointestinal tract on the other. Compared to intravenous and oral administrations, the variety of carriers for transdermal applications of drugs is significantly smaller and practically limited to nanoemulsions [17] emulsions, [17] and gels [18].

A variation to the simple transdermal administration is iontophoresis. In this method the penetration of ionic drugs through the skin is promoted by application of low voltage with continuous constant current [19]. Hydrophilic macromolecules require a higher voltage, and the method is then called electroporation [20]. Current promoted administrations are typically applied for topical therapy of head and neck cancers [21].

ANTICANCER DRUGS CARRIERS**Objectives for drug carriers**

Drug carriers can be multifunctional, which means that they provide benefits in more than one aspect. A generic function is the protection of the drug from untimely degradation inside the human body. This, particularly, applies for the period the drug

requires to reach the targeted action site. The protection of the drug molecule is accompanied by protection of non-targeted tissue from potential damage by the drug. This aspect, aiming on a minimization of side effects of the medical treatment, becomes a key feature for targeting delivery systems, which can be compared with a postal delivery of a bomb that requires opening of the package to cause damage. Unfortunately targeted drug delivery is currently a subject of research but far away from application [22,23].

Besides the protection of the drug, the carrier can aim for a constant release of the drug over a period of time. This way a steady drug concentration may be achieved without frequent drug administrations. At the same time the approach avoids, or at least minimizes, high drug concentrations, which typically increase side effects. The drug carrier in such a case acts as a reservoir or 'release system' to provide an ideally constant concentration of active ingredient [22,23].

Finally, the carrier can serve to provide passage to the targeted site of action. Since anticancer drugs commonly act inside the cell, they need to pass the cellular membrane prior to any action. Although nature provides mechanisms for exchange processes between cells and the environment, a carrier that actively promotes bypassing of the cellular membrane potentially enhances the efficiency of the drug [22-24].

Hydrogels

Hydrogels are three-dimensional assemblies of hydrophilic polymers. They are either cross-linked thermoset gels or high molecular weight (plastic) polymers that are formulated into a colloidal assembly. As the name suggests, they exhibit strong affinity for water. The crosslinking or high molecular weight, however, prevents them to dissolve; instead they swell, thus providing a sponge like structure. The swelling of hydrogels can lead to a water content exceeding 90% of the gel [22]. The porous structure provides cavities for the loading with drugs. The latter can subsequently diffuse out of the hydrogel carrier and ideally ensures a steady concentration of bio-accessible drug. The function of a hydrogel, hence, is that of a reservoir releasing the drug continuously. An example for the use of hydrogels in cancer therapy is the proposed co-delivery of

metformin and 5-fluorouracil using a Schiff base-based hydrogel for the treatment of colon cancer [23].

Poly(hydroxycarboxylic acids)

Poly(hydroxycarboxylic acids), also termed PHAs, are biodegradable polymers. Typical examples are PLA, or poly lactic acid, and PLGA, a copolymer of lactic and glycolic acid [24]. The ester linkage of these polymers is susceptible to biological hydrolysis, giving rise to a slow release of enwrapped drugs. Like hydrogels, PHAs aim for a steady drug level inside the body. However instead of a diffusion process, the drug release relies on a lipase induced degradation of the polymer matrix. More recently longer chained PHAs, particularly medium chain PHAs, have gained interest [25]. The choice of the monomers potentially enables a tuning of the degradation rate and, hence, the release. Besides, it also may affect the drug loading efficiency of the polymer. Examples for the application of PHAs in drug delivery are coated microsphere formulations for the treatment of cerebral tumors. For 5-fluorouracil containing carriers a chitosan coating was applied [26], while a taxol containing carrier was coated with polyethylene glycol instead [27].

Nanotubes

Nanotubes provide an internal volume, which can be filled with drug molecules. The function of nanotubes in drug delivery is closely related to the hydrogel, i.e. providing a reservoir for the release of the drug. The radius of the nanotube must be sufficiently large to enable the active ingredient to diffuse into the interior, while the interior volume limits the maximum drug uptake. The nanotubes have different inner and outer surfaces for functionalization, potentially affecting the interaction with the drug and the host organism, respectively. Inorganic-based nanotubes can be either single walled [28] based on carbon, boron carbide, boron nitride and silicon, or multi-walled carbon nanotubes [29]. Organic variants are hollow cylindrical nanomaterials consisting of monomer units, containing both hydrophilic and hydrophobic domains. The unique structures of these amphiphilic molecules drive their self-assembly in aqueous media [30]. A hydrophobic, poorly water-soluble drug, such as hydrocortisone, was encapsulated using organic nanotubes [31], whereas for a hydrophilic drug, i.e. 5-

fluorouracil, nanotubes based on TiO_2/ZnS were applied [32].

Nanoparticles

Nanoparticles are tentatively spherical structures with a size ranging from 1-100 nm in all dimension. Common nanoparticle drug carriers cover solid lipid nanoparticles [33] as well as polymeric nanoparticles [34]. Both, natural and synthetic, polymers have been applied; examples cover cellulose and chitosan on the one hand and acrylic polymers on the other. The coating of a drug can prevent its degradation under the acidic conditions inside the stomach, while it can subsequently be released in the distal ileum. Nanoparticulate coating is, therefore, particularly interesting for oral administration. The approach has been applied on paclitaxel, a hydrophobic drug used for treating ovarian, breast, lung, pancreatic and other cancers. Paclitaxel loaded lipid-based nanoparticles enhanced the drug availability at the tumour compared to application of the non-coated drug. [35]. 5 Fluorouracil, a hydrophilic drug, on the other hand, has been successfully formulated into polymeric nanoparticles using chitosan. The nanoparticles effectively retained the drug loading at acidic conditions, but released it upon reaching mild alkaline. Most of the drug was released within a few hours [36].

Recently graphene, or more typically graphene oxide, has been applied in the preparation of drug-loaded nanoparticles [37]. The aromatic system in grapheme derivatives enables the binding of drug molecules via π - π -stacking [38]. Moreover, chemical modification of the grapheme oxide potentially enables a targeting drug delivery [39]. The functionalization of this sheet-shaped material with cyclodextrins as organic hosts for small organic molecules, like most drugs, is particularly promising [40]. Nanoparticles can be obtained by crosslinking functionalized graphene sheets [41]. The active incorporation of the drug inside the graphene bonded supramolecular hosts in combination with an effective crosscoupling potentially enables a high drug loading, thus providing advantages over other nanoparticle carriers.

Vesicles and Liposomes

Vesicle is a universal term for a self-enclosed bilayer amphiphilic. Inside the enclosed aqueous phase of the vesicle water-soluble drugs can be encapsulated, while oil-soluble

drugs may be incorporated into the core of the hydrophobic bilayer [31,42,43]. In other words, vesicles enable the encapsulation and controlled release of both, hydrophilic and hydrophobic, active ingredients. They find applications not only in drug delivery systems, but also for agrochemicals and personal care products. Unlike the previously discussed drug carriers, vesicles potentially promote an active delivery of the drug into a cell similar to cellular exchange processes involving membrane fusion and separation [36,44,45].

Vesicles are classified according to size and lamellarity, reflecting the number of bilayers separating the aqueous core from the water environment. An illustration of the most common vesicle types and their preparation is provided in (Figure 1). Typically vesicles adopt a spherical shape. Ultra-small unilamellar vesicle (USUV) having diameters in a range of 5-10 nm, [39,46] whereas the common size for small unilamellar vesicle (SUV) is almost a magnitude larger. Reported criteria vary from 8-40 nm, [41] over 20-50

nm, [47] up to <100 nm in diameter [48]. These vesicles, which all contain a single bilayer only, can be prepared either by the solvent injection method [49] or by sonication and extrusion of large vesicle precursors, respectively. The latter covers large unilamellar vesicles (LUV) and multilamellar vesicles (MLV). LUVs typically have a diameter exceeding 100 nm, or 0.1 μm [48,50] and can reach a size of several μm [51]. Like SUV they can be obtained by a solvent injection method, usually using either ether or ethanol [48]. MLVs have similar [48] or even larger sizes [50] than LUV, but consist of two or more concentric bilayers [51,52]. Their preparation applies thin film hydration [48] or freeze-drying of preformed SUV dispersions in an aqueous solution of the drug to be encapsulated [48]. The biggest vesicles are called giant vesicles (GUV), covering a range of about 5-100 μm [51-53], whereas the last category, oligovesicular vesicles (OVV), indicate small vesicles incorporated inside big vesicles [54].

INJECTION METHOD

THIN FILM HYDRATION

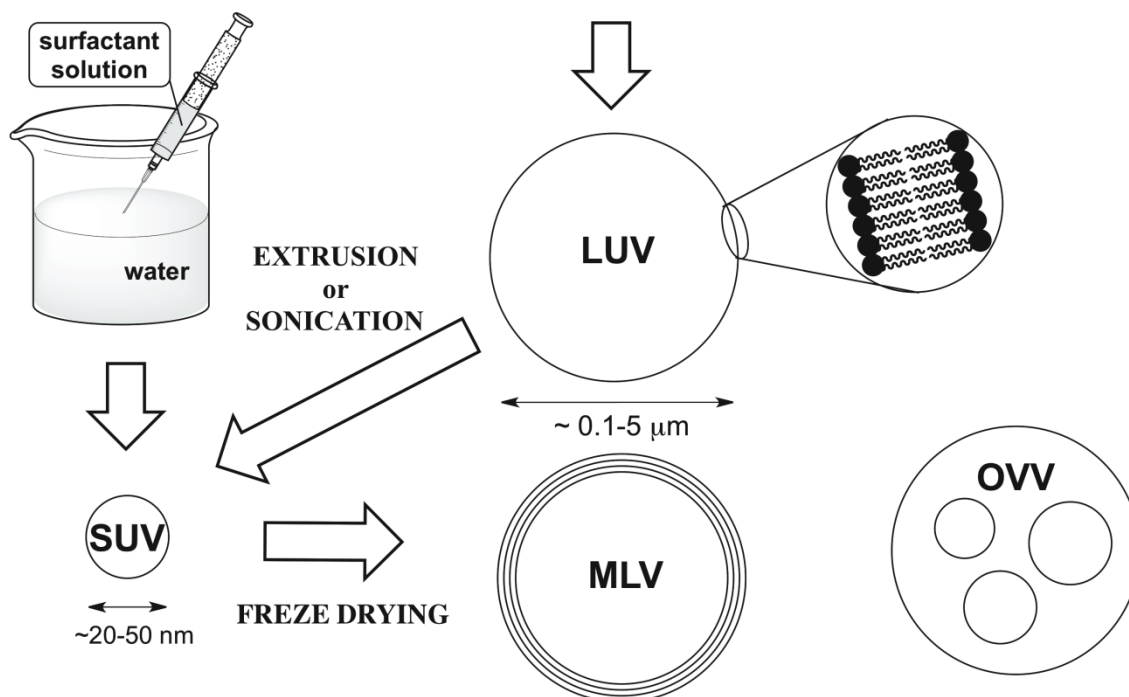


Figure 1: Overview on typical vesicle types and their preparation.

SUV are commonly used for intravenous injection [55]. This application is limited to sizes below 100 nm [52] and determined by the diameter of the average blood capillary, which is 7000 nm [51]. Although blood cells with a diameter of 8000 nm can pass these capillaries, vesicles are less flexible and, Hussen RSD et al. IJPRR2016;5(4)

hence, must be smaller to ensure effective circulation within the blood stream. There are many synonyms for vesicles, reflecting the base material used: [51] Liposomes are very common lipid vesicles, primarily prepared from phospholipids [49,50,56], while niosomes apply non-ionic surfactants

[57] and catanionic vesicles, or “catansomes” are formed by pairing anionic and cationic surfactants in equimolar ratio [58]. Finally, the term ethosomes refers to phospholipid vesicular systems embodying ethanol in high concentrations, i.e. about 20-50 wt% [59].

CHARACTERIZATION OF DRUG CARRIER

Physico-chemical characterization

The average size and its distribution, typically reported as polydispersity index, can be determined using dynamic light scattering [60]. This method is generic and can be applied for a variety of drug carriers, provided a homogeneous liquid phase can be obtained. More information regarding the carrier morphology, reflecting surface roughness and shape of a drug carrier can be obtained using field emission scanning electron microscopy (FESEM) or transmission electron microscopy (TEM) [61]. Vesicular systems commonly require specialized equipment for TEM, also referred to as cryoTEM [62].

The surface charge is an important feature, since it determines the interaction of the drug carrier with its environment, including the surfaces of potential cellular targets. It is typically measured as zeta potential. Zeta potential measurements have a high significance in the preparation and optimization of drug carriers, as the magnitude enables to predict the stability of a colloidal formulation. High zeta potentials indicate strong repulsive interactions of drug carriers, which stabilize a colloidal dispersion, while the risk of coagulation or flocculation increases with low zeta potential. For typical colloidal systems zeta potentials between 0 mV to ± 5 mV refers to rapid coagulation or flocculation, while a range between 10 mV to 30 mV, positive or negative, indicates incipient instability. Moderate stability is obtained with zeta potentials between 30 mV and 40 mV, whereas good stability requires a range from 40 mV to 60 mV. Zeta potentials exceeding 60 mV are associated with excellent dispersion stability [63,64].

Encapsulation efficiency

The anticancer drug loading of carriers can be monitored by various techniques that enable the determination of concentration of the respective drug. Typical methods are UV-VIS spectroscopy, [65] fluorescence spectroscopy, [66] and HPLC [67]. Combination of the latter with mass

spectrometry [59] avoids misinterpretations due to additives. The drug encapsulation efficiency requires the separation of free from encapsulated drug. The latter can be achieved by ultrafiltration, e.g. by using centrifugal filter devices [60]. Besides, specific physical properties of the carrier, like magnetic behaviour, can be applied as well [61]. The amounts of non-encapsulated drug in the supernatant and encapsulated drug in the sediment are separately determined based on suitable calibration curves.

The drug encapsulation efficiency refers to the share of the utilized drug that is encapsulated inside the carrier. Typically mass fractions are applied, reported as EE%. The encapsulation efficiency varies widely with the material and the method utilized for the carrier preparation. The following data, reflecting the encapsulation of 5-Fluorouracil in a variety of carriers illustrate this nicely: Application of a reverse-phase evaporation approach the vesicular encapsulation efficiency of a system containing of dipalmitoylphosphatidyl choline (DPPC) and cholesterol system led to an EE% of 5.7%. This encapsulation efficiency was about one magnitude higher compared to the same system when an ethanolic injection method was applied [68]. The difference probably reflects a higher dilution factor for the injection method. On the other hand, application of DPPC without cholesterol in a modified reverse-phase evaporation approach increased the encapsulation efficiency to above 90%, [69] and the use of a different surfactant system enabled an EE% of about 80% despite the use of an injection method [70]. Compared to the vesicular encapsulation, the use of polymeric nanoparticles based on triphosphate-crosslinked chitosan was with ~70% and 42-55%, respectively, less effective [71,72].

***In vitro* drug release**

Even more important than the encapsulation efficiency is the release profile of the drug. In fact, it is the most important consideration for a delivery system, since it controls the bioavailability and, hence, the therapeutic efficacy. The most popular and common method to study the *in vitro* drug release is the so-called dialysis bag method. In this method the encapsulated drug is placed inside dialysis bag into a bulk volume of moving water at 37°C, mimicking a body fluid. The drug concentration is determined

at various time intervals. Variations of the dialysis bag method cover side-by-side diffusion cells as well as ultra-centrifugations and ultra-filtration approaches [73-76].

CONCLUSION

Drug delivery is a complex matter with many facets. A variety of drug carriers is applied catering for three major administration routes. Presently most common are release systems, aiming for a steady concentration of the drug, whereas the ultimate goal of a targeted drug delivery system remains at an early stage of development. Owing to their encapsulation ability for both, hydrophilic and hydrophobic, drugs, vesicular delivery systems are considered most promising. Moreover, they potentially enable the incorporation of recognising domains, thus providing access to targeted drug delivery as well. The probably best competitors are nanoparticle incorporating graphene oxide, as they potentially provide similar features. However, unlike vesicles, the current nanoparticles lack the potential to promote the penetration of the cellular membrane. On the other hand, nanoparticles provide potential advantages in terms of stability, owing to a wider range of material to be applied. Therefore nanoparticles are considered better candidates for oral delivery systems compared to vesicles.

REFERENCES

- Sharma S, Doherty KM, Brosh RM Jr. DNA helicases as targets for anti-cancer drugs. *Curr Med Chem Anticancer Agents*. 2005; 5(3): 183-199.
- Berdis AJ. DNA Polymerases as Therapeutic Targets. *Biochemistry*. 2008; 47(32): 8253-8260.
- Cheung-Ong K, Guri Giaever G and Nislow C. DNA-Damaging Agents in Cancer Chemotherapy: Serendipity and Chemical Biology. 2013; 20(5): 648-659.
- Grattan BJ Jr. Plant Sterols as Anticancer Nutrients: Evidence for Their Role in Breast Cancer. *Nutrients*. 2013; 5(2): 359-387.
- Rodriguez-Ruiz V, Maksimenko A, Anand R, Monti S, Agostoni V, Couvreur P et al. Efficient "green" encapsulation of a highly hydrophilic anticancer drug in metal-organic framework nanoparticles. *J Drug Target*. 2015; 23(7-8): 759-767.
- Ma Y, Liu D, Wang D, Wang Y, Fu Q, Fallon JK et al. Combinational Delivery of Hydrophobic and Hydrophilic Anticancer Drugs in Single Nanoemulsions To Treat MDR in Cancer. *Mol. Pharmaceutics*. 2014; 11 (8): 2623-2630.
- Saville MW, Lietzau J, Pluda JM, Wilson WH, Humphrey RW, Feigel E, et al. Treatment of HIV-associated Kaposi's sarcoma with paclitaxel. *The Lancet*. 1995; 346 (8966): 26-28.
- Remesh A. Toxicities of anticancer drugs and its management. *Int J Basic Clin Pharmacol*. 2012; 1(1): 2-12.
- Mo Y, Wang H, Liu J, Lan Y, Guo R, Zhang Y, et al. Controlled release and targeted delivery to cancer cells of doxorubicin from polysaccharide-functionalised single-walled carbon nanotubes. *J. Mater. Chem. B*. 2015; 3: 1846-1855.
- Dufes C, Muller JM, Couet W, Olivier JC, Uchegbu IF, Schätzlein AG. Anticancer drug delivery with transferrin targeted polymeric chitosan vesicles. *Pharm Res*. 2004; 21(1): 101-107.
- Teicher BA, Holden SA, Ara G, Ha CS, Herman TS, Northey D. A new concentrated perfluorochemical emulsion and carbogen breathing as an adjuvant to treatment with antitumor alkylating agents. *J Cancer Res Clin Oncol*. 1992; 118(7): 509-514.
- Kalepu S, Manthina M, Padavala V. Oral lipid-based drug delivery systems: an overview. *Acta Pharmaceutica Sinica B*. 2013; 3(6): 361-372.
- Shaji KP, Umesha S, Salimath BP. A novel liquid oral formulation for 1-Octacosanol an anticancer drug and its stability study. *Indian Journal of Research in Pharmacy and Biotechnology*. 2015; 3(3): 186-191.
- Arias J L. Novel Strategies to Improve the Anticancer Action of 5-Fluorouracil by Using Drug Delivery Systems. *Molecules*. 2008; 13: 2340-2369.
- Hua S. Orally administered liposomal formulations for colon targeted drug delivery. *Front Pharmacol*. 2014; 5: 1-4.
- Cheng Q, Shi H, Huang H, Cao Z, Wang J, Liu Y. Oral delivery of a platinum anticancer drug using lipid assisted polymeric nanoparticles. *Chem. Commun*. 2015; 51: 17536-17539.
- ElMeshad AN, Tadros MI. Transdermal Delivery of an Anti-Cancer Drug via W/O Emulsions Based on Alkyl Polyglycosides and Lecithin: Design, Characterization, and In Vivo Evaluation of the Possible

- Irritation Potential in Rats. *AAPS PharmSciTech*. 2011; 12(1): 1-9.
18. Elnaggar YS, El-Refaie WM, El-Massik MA, Abdallah OY. Lecithin-based nanostructured gels for skin delivery: an update on state of art and recent applications. *J Control Release*. 2014; 180: 10-24.
 19. Prausnitz MR. A practical assessment of transdermal drug delivery by skin electroporation. *Advanced Drug Delivery Reviews*. 1999; 35: 61-76.
 20. Banga AK, Bose S, Ghosh TK. Iontophoresis and electroporation: comparisons and contrasts. *Int J Pharm*. 1999; 179: 1-19.
 21. Gratieri T, Kalia YN. Targeted local simultaneous iontophoresis of chemotherapeutics for topical therapy of head and neck cancers. *Int J Pharm*. 2014; 460: 24-27.
 22. Hoare TR, Kohane DS. Hydrogels in drug delivery: Progress and challenges. *Polymer*. 2008; 49: 1993-2007.
 23. Wu X, He C, Wu Y, Chen X. Synergistic therapeutic effects of Schiff's base cross-linked injectable hydrogels for local co-delivery of metformin and 5-fluorouracil in a mouse colon carcinoma model. *Biomaterials*. 2016; 75: 148-162.
 24. Anderson JM, Shive MS. Biodegradation and biocompatibility of PLA and PLGA microspheres. *Adv Drug Deliv Rev*. 1997; 28: 5-24.
 25. Wang Z, Itoh Y, Hosaka Y, Kobayashi I, Nakano Y, Maeda I, Yag K. Novel transdermal drug delivery system with polyhydroxyalkanoate and starburst polyamidoamine dendrimer. *J. Biosci. Bioeng* 2003; 95(5): 541-543.
 26. Chandy T, Das GS, Rao GH. 5-Fluorouracil-loaded chitosan coated polylactic acid microspheres as biodegradable drug carriers for cerebral tumours. *J Microencapsul*. 2000; 17(5): 625-638.
 27. Das GS, Rao GHR, Wilson RF, Chandy T. Controlled delivery of taxol from poly(ethylene glycol)-coated poly(lactic acid) microspheres. *J Biomed Mater Res*. 2001; 55(1): 96-103.
 28. Yao HJ, Sun L, Liu Y, Jiang S, Pu Y, Li J et al. Monodistearoylphosphatidylethanolamine-hyaluronic acid functionalization of single-walled carbon nanotubes for targeting intracellular drug delivery to overcome multidrug resistance of cancer cells. *Carbon*. 2016; 96: 362-376.
 29. Li J, Pant A, Chin CF, Ang WH, Ménard-Moyon C, Nayak TR et al. In vivo biodistribution of platinum-based drugs encapsulated into multi-walled carbon nanotubes. *Nanomedicine: Nanotechnology, Biology, and Medicine*. 2014; 10: 1465-1475.
 30. Moribe K, Makishima T, Higashi K, Liu N, Limwikrant W, Ding W et al. Encapsulation of poorly water-soluble drugs into organic nanotubes for improving drug dissolution. *Int J Pharm*. 2014; 469: 190-196.
 31. Da Silveira NP, Mertins O, Sebben M, Pohlmann AR. Production of soybean phosphatidylcholine-chitosan nanovesicle by reversed phase evaporation: a step by step study. *Chemistry and Physics of Lipids*. 2005; 138: 29-37.
 32. Faria HAM, De Queiroz AAA. A novel drug delivery of 5-fluorouracil device based on TiO₂/ZnS nanotubes. *Materials Science and Engineering C*. 2015; 56: 260-268.
 33. Al-Ajmi MF, Hussain A, Ahmed F. Novel synthesis of ZnO nanoparticles and their enhanced anticancer activity: Role of ZnO as a drug carrier. *Ceramics International*. 2016; 42: 4462-4469.
 34. Tummala S, Kumar SMN, Prakash A. Formulation and characterization of 5-Fluorouracil enteric coated nanoparticles for sustained and localized release in treating colorectal cancer. *Saudi Pharmaceutical Journal*. 2015; 23: 308-314.
 35. Liu Y, Zhang B, Yan B. Enabling Anticancer Therapeutics by Nanoparticle Carriers: The Delivery of Paclitaxel. *Int. J. Mol. Sci*. 2011; 12: 4395-4413.
 36. Martens d S, McMahon HT. Mechanisms of membrane fusion: disparate layers and common principles. *Nature Reviews Molecular Cell Biology*. 2008; 9: 543-556.
 37. Chaudhary K. Graphene, its synthesis and its application in anti-cancer drug delivery. *International Journal of Science and Research*. 2015; 4(8): 741-744.
 38. Zhang L, Xia J, Zhao Q, Liu L, Zhang Z. Functional graphene oxide as nanocarrier for controlled loading and targeted delivery of mixed anticancer drugs. *Small* 2006; 6(4): 537-544.

39. Kühnle A, Fernandez P, Willenbacher N, Frechen T. Vesicles as rheology modifier. *Colloids and Surfaces A: Physicochem. Eng. Aspects.* 2005; 262: 204-210.
40. Tan J, Meng N, Fan Y, Su Y, Zhang M, Xiao Y et al. Hydroxypropyl- β -cyclodextrin-graphene oxide conjugates: Carriers for anti-cancer drugs. *Materials Science and Engineering C.* 2016; 61: 681-687.
41. Ostrowsky N, Sornette D. Interactions between nonionic vesicles. Importance of the aliphatic-chain phase transition. In: V. Degiorgio and M. Corti, Editors, *Physics of amphiphiles: micelles, vesicles and microemulsions*, Elsevier Science, Bologna. 1985; pp. 563-586.
42. Polozova A, Yamazaki A, Brash JL, Winnik FM. Effect of polymer architecture on the interactions of hydrophobically-modified poly-(N-isopropylamides) and liposomes. *Colloids Surf. A.* 1999; 147: 17-25.
43. Sharma A, Sharma US. Liposome in drug delivery: progress and limitations. *Int. J. Pharm.* 1997; 154: 123-140.
44. Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P. *Molecular Biology of the Cell.* 4th ed. New York: Garland Science. 2002.
45. Cevc G, Richardsen H. Lipid vesicles and membrane fusion. *Advanced Drug Delivery Reviews.* 1999; 38(3): 207-232.
46. Lionberger RA, Russel WB. High frequency modulus of hard sphere colloids. *J. Rheol.* 1994; 38: 1885-1908.
47. Vemuri S, Rhodes. CT. Preparation and Characterization of liposomes as therapeutic delivery systems: a review. *Pharmaceutica Acta Helvetiae.* 1995; 70: 95-111.
48. Ohsawa T, Miura H, Harada K, A novel method for preparing liposome with a high capacity to encapsulate proteinous drugs: freeze-drying method. *Chem. Pharm. Bull.* 1984; 32: 2442-2445.
49. Batzri S, Korn ED. Single bilayer liposomes prepared without sonication. *Biochim. Biophys. Acta.* 1973; 298: 1015-1019.
50. Honeywell-Nguyen PL, Bouwstra JA. *Drug Discovery Today: Technologies. Drug delivery/formulation and nanotechnology.* 2005; 2: 67-74.
51. Fuchs J, Podda M. *Encyclopedia of medical genomics and proteomics.* New York: Marcel Dekker. 2005.
52. Sherwood L. *Human physiology: From cells to systems.* (7th edn). Cengage Learning. 2008.
53. Gradzielski M, Muller M, Bergmeier M, Hoffmann H, Hoinkis E. *J. Phys. Chem.* 1999; 103: 1416-1424.
54. Šegota S, Težak Đ. Spontaneous formation of vesicles. *Advances in Colloid and Interface Science.* 2006; 121: 51-75.
55. Göppert TM, Müller RH. Polysorbate-stabilized solid lipid nanoparticles as colloidal carriers for intravenous targeting of drugs to the brain: comparison of plasma protein adsorption patterns. *J Drug Target.* 2005; 13(3): 179-187.
56. Bangham AD, Standish MM, Watkins JC. Diffusion of univalent ions across the lamellae of swollen phospholipids, *J. Mol. Biol.* 1965; 13: 238-252.
57. Uchegbu IF, Vyas SP. Non-ionic surfactant based vesicles (niosomes) in drug delivery. *Int. J. Pharm.* 1998; 172: 33-70.
58. Yang YM, Wu KC, Huang ZL, Chang CH, Chou TH. Enhancement of catansome formation by means of cosolvent effect: Semi-spontaneous preparation method. *Colloids and Surfaces A: Physicochem. Eng. Aspects.* 2007; 302: 599-607.
59. Alshamsan A. Nanoprecipitation is more efficient than emulsion solvent evaporation method to encapsulate cucurbitacin I in PLGA nanoparticles. *Saudi Pharmaceutical Journal.* 2014; 22(3): 219-222.
60. Sharma V, Anandhakumar S, Sasidharan M. Self-degrading niosomes for encapsulation of hydrophilic and hydrophobic drugs: An efficient carrier for cancer multi-drug delivery. *Materials Science and Engineering C.* 2015; 56: 393-400.
61. Dutta S, Parida S, Maiti C, Banerjee R, Mandal M, Dhara D. Polymer grafted magnetic nanoparticles for delivery of anticancer drug at lower pH and elevated temperature. *J. Colloid. Interface. Sci.* 2016; 467: 70-80.
62. Won YY, Brannan AK, Davis HT, Bates FS. Cryogenic transmission electron microscopy (Cryo-TEM) of micelles and vesicles formed in water by poly(ethylene oxide)-based block copolymers. *J. Phys. Chem. B.* 2002; 106(13): 3354-3364.

63. Greenwood R, Kendall K. Electroacoustic studies of moderately concentrated colloidal suspensions. *J. Eur. Ceram. Soc.* 1999; 19(4): 479-488.
64. Hanaor DAH, Michelazzi M, Leonelli C, Sorrell CC. The effects of carboxylic acids on the aqueous dispersion and electrophoretic deposition of ZrO₂. *J. Eur. Ceram. Soc.* 2012; 32(1): 235-244.
65. Panwar P, Pandey B, Lakhera P C, Singh KP. Preparation, characterization, and in vitro release study of albendazole-encapsulated nanosize liposomes. *Int. J. Nanomedicine.* 2010; 5: 101-108.
66. Sadatmousavi P, Chen P. Self/Co-Assembling Peptide, EAR8-II, as a Potential Carrier for a Hydrophobic Anticancer Drug Pirarubicin (THP)—Characterization and in-Vitro Delivery. *Int J Mol Sci.* 2013; 14(12): 23315-23329.
67. López-Gasco P, Iglesias I, Benedí J, Lozano R, Blanco MD. Characterization and in-vitro bioactivity evaluation of paclitaxel-loaded polyester nanoparticles. *Anticancer Drugs.* 2012; 23(9): 947-958.
68. Da Costa CAM, Moraes ÂM. Encapsulation of 5-fluorouracil in liposomes for topical administration. *Acta Scientiarum Technology Maringá.* 2003; 25: 53-61.
69. Pentak D, Sułkowski WW, Sułkowska A. Influence of some physical properties of 5-fluorouracil on encapsulation efficiency in liposomes. *J. Therm. Anal. Calorim.* 2012; 108: 67-71.
70. Mak OW, Seman N, Harun NH, Hussen RSD, Mohd Rodzi N, Heidelberg T. Nanovesicles based on mixtures of a biantennary glycolipid with ionic co-surfactants. *J. Surfactants. Deterg.* 2015; 18: 973-980.
71. Aydin RST, Pulat M. 5-Fluorouracil Encapsulated Chitosan Nanoparticles for pH-Stimulated Drug Delivery: Evaluation of Controlled Release Kinetics. *J. Nanomater.* 2012; 2012: 1-10.
72. Honary S, Ebrahimi P, Hadianamrei R. Optimization of size and encapsulation efficiency of 5-FU loaded chitosan nanoparticles by response surface methodology. *Curr Drug Deliv.* 2013; 10: 742-752.
73. Singh R, Lillard Jr. JW. Nanoparticle-based targeted drug delivery. *Exp Mol Pathol.* 2009; 86(3): 215-223.
74. Anitha A, Deepagan VG, Divya Rani VV, Menon D, Nair SV, Jayakumar R. Preparation, characterization, in vitro drug release and biological studies of curcumin loaded dextran sulphate-chitosan nanoparticles. *Carbohydrate Polymers.* 2011; 84(3): 1158-1164.
75. Fu Y, Kao WJ. Drug Release Kinetics and Transport Mechanisms of Non-degradable and Degradable Polymeric Delivery Systems. *Expert Opin Drug Deliv.* 2010; 7(4): 429-444.
76. Sahoo SK, Sahoo SK, Behera A, Patil SV, Panda SK. Formulation, in vitro drug release study and anticancer activity of 5-fluorouracil loaded gellan gum microbeads. *Acta Pol Pharm.* 2013; 70(1): 123-127.