REVIEW

Ph.D. Program in Nanoscience and Technology (International)[1], Graduate School; Metallurgy and Materials Science Research Institute[2], Chulalongkorn University, Bangkok, Thailand

Biopolymeric alginate-chitosan nanoparticles as drug delivery carriers for cancer therapy

S. Bhunchu[1], P. Rojsitthisak[2]

Received July 21, 2013, accepted November 22, 2013

Pranee Rojsitthisak, Metallurgy and Materials Science Research Institute, Chulalongkorn University, Bangkok 10330, Thailand
Pranee.L@chula.ac.th

Pharmazie 69: 563–570 (2014) *doi: 10.1691/ph.2014.3165*

Nanoparticulate drug delivery systems enhance cancer treatment by direct entry of nanometer particles into the fenestration in the vasculature of cancer cells. Nanoparticles for encapsulation of anticancer drugs are preferably prepared using natural polymers as carriers, with polysaccharides being particularly favorable. Alginate and chitosan polysaccharides have been widely used in nanoparticulate drug delivery systems because of their biodegradable, biocompatible, non-toxic and bioadhesive properties. In this review, we present an overview of drug delivery systems for cancer treatment, describe the use of biopolymeric alginate-chitosan nanoparticles for anticancer drug delivery, and discuss the important characteristics of these nanoparticles for use in drug delivery.

1. Introduction

Surgery, chemotherapy and radiation are widely used for cancer therapy, but have limitations such as destruction of healthy cells, cytotoxicity, inflammatory effects, and skin burning from radiation (Decker-Baumann et al. 1999; Subramani 2009).

A nanoparticulate drug delivery system (NPDDS) offers an alternative approach to delivery of anticancer drugs that may improve their pharmacological and therapeutic properties (Chellat et al. 2005; Haley and Frenkel 2008; Singh and Lillard Jr 2009; Subramani 2009). A NPDDS can protect an anticancer drug against degradation during delivery to the target tissue and also provide a therapeutic level of the drug at specific organ sites. Various types of nanoparticles have been used in drug delivery systems, including polymeric nanoparticles, nanovesicles and nanoemulsions (Fig. 1) (Mohanraj and Chen 2006). In such nanoparticles, the use of natural biopolymers (and especially polysaccharides) is preferable owing to their non-toxicity, biodegradability, biocompatibility, and protective and hydrophilic properties (Kumari et al. 2010). Biopolymeric hydrogel nanoparticles are formulated by an interaction between anionic and cationic biopolymers (Gazori et al. 2009; Lertsutthiwong and Rojsitthisak 2011; Li et al. 2008; Malesu et al. 2011). The hydrogel nanoparticles have good characteristics for encapsulation of drugs and delivery to a target site (Das et al. 2010; Douglas and Tabrizian 2005, Motwani et al. 2008; Lertsutthiwong et al. 2009).

Most attempts at use of anticancer drugs in a drug delivery system have involved hydrophobic drugs encapsulated into an aqueous nanoparticulate system, with the goal of delivering the drug to the target site and releasing the full potential of the encapsulated drug (Kumar 2000). The biodegradable, biocompatible and non-toxic properties required for the polymers in these systems are met by natural biopolymers such as alginate and chitosan. In this review, we provide an overview of the use of drug delivery systems in cancer treatment and of biopolymeric alginate-chitosan nanoparticles as anticancer drug carriers. We also discuss the important characteristics of alginate-chitosan nanoparticles for use in a drug delivery system.

2. Drug delivery systems for cancer treatment

Cancer is a group of diseases related to abnormal cell growth and uncontrollable cell division. Cancer cells can also spread to other parts of the body *via* the blood stream and lymphatic system, and consequently destroy healthy cells (American Cancer Society 2011). Anand et al. (2008) reported that 90–95% of cancers occur due to environmental pollutants, radiation, infection, tobacco use, poor diet and obesity, and 5–10% due to genetics. Surgery is the primary treatment for all types of cancer, but is not suitable when cancer cells have spread to other parts of the body. Side effects of pain, fatigue, bleeding, infection and lymphedema may also occur. Chemotherapy and radiotherapy also have side effects such as destruction of healthy cells, toxicity and loss of hair (Decker-Baumann et al. 1999; Mittelberg et al. 1996). Therefore, new cancer therapies are required and there is growing interest in NPDDS development for delivery of anticancer drugs to a target organ.

A drug delivery system is used for administration of a drug to achieve a therapeutic effect with safety and convenience to patients (Kumar et al. 2000). A NPDDS is a modified drug delivery system using nanotechnology for (i) improvement of specific drug targeting and delivery efficiency, (ii) reduction of side effects, (iii) improved safety and biocompatibility, and (iv) faster and lasting development of medicines (Singh and Lillard Jr 2009; Subramani 2009). NPDDSs have been widely studied for anticancer drugs (Gelperina et al. 2005; Kukowska-Latallo et al. 2005; Pandey and Khuller 2006) because of their versatile properties. For example, nanoparticles of size 10–100 nm are suitable for intravenous delivery because the nanoparticles are smaller than the diameter of intravenous capillaries (5–6 μm),

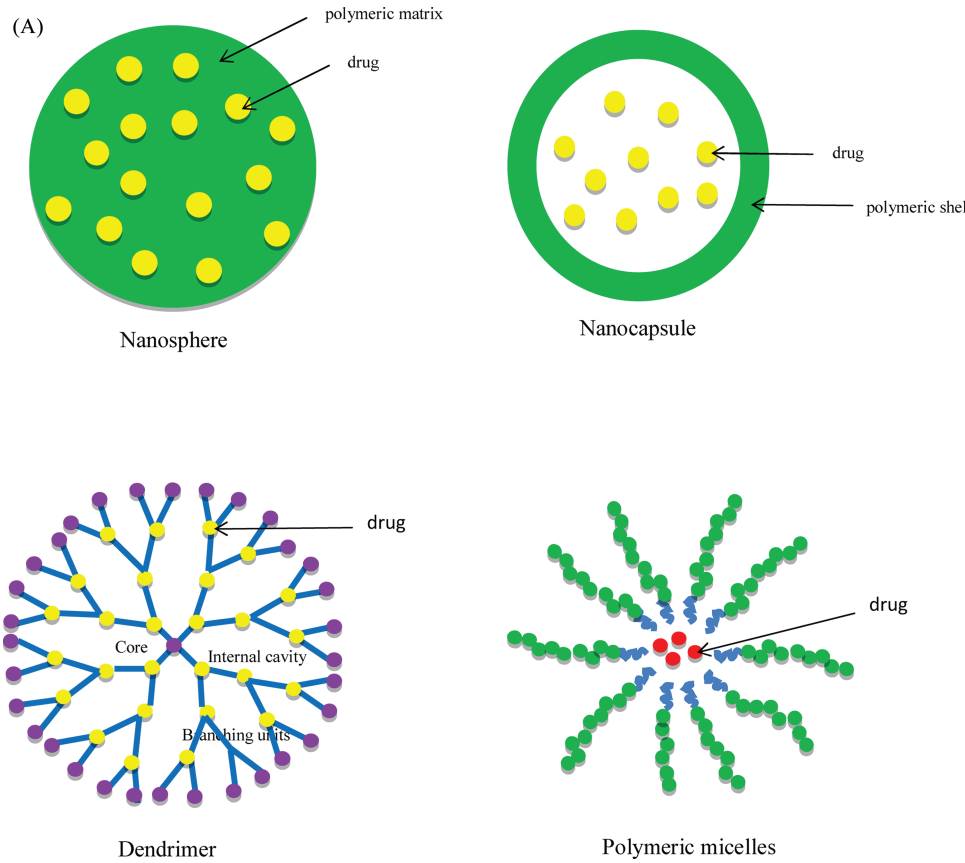


Fig. 1: Types of nanoparticles used in nanoparticulate drug delivery systems: (a) polymeric nanoparticles, (b) nanovesicles and (c) nanoemulsions.

and thus can pass through the blood circulation and distribute *in vivo* (Davis 2006; Juillerat-Jeanneret 2008). Nanoparticles can also penetrate through tissues to approach sites that larger particles cannot reach. For example, Bawa (2008) reported that chemotherapeutic drug-nanoparticles can be delivered into tumor cells *via* leaky holes in the microvasculature, with an extended circulation time and accumulation of the drug within the tumor cells.

Nanoparticles can be classified as natural or synthetic (Oberdörster et al. 2005; Singh and Lillard Jr 2009). Examples of synthetic nanoparticles include carbon black, carbon nanotubes, fullerenes, quantum dots, metals, metal oxides and semiconductors. However, synthetic nanoparticles may be toxic to humans, animals and the environment (Colvin 2003; Derfus et al. 2004), and thus natural nanoparticles are used for biomedical applications. Various biopolymers have been used in such nanoparticles, including poly(3-hydroxybutyrate), poly(lactic acid), poly(e-caprolactone), and copolymers of polyglycolide, alginate and chitosan (Shukla and Tiwari 2012). Examples of nanoparticles produced from various biopolymers for cancer treatment are shown in Table 1. Among these biopolymers, chitosan and alginate are of particular interest for drug delivery to cancer cells because of their biodegradable, biocompatible, hydrophilic, mucoadhesive and protective properties (Chellat et al. 2005; Coppi and Iannuccelli 2009; Jayakumar et al. 2010; Shukla and Tiwari 2012).

3. Biopolymeric alginate-chitosan nanoparticles as anticancer drug delivery systems

3.1. Alginate

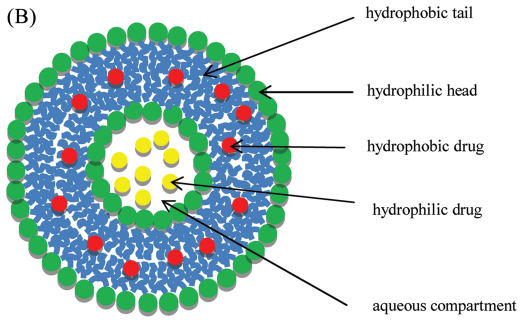
Alginate is an anionic polysaccharide consisting of linear copolymers of α-L-guluronate and β-D-mannuronate residues linked by (1–4) glycosidic linkages (Fig. 2) (Lertsutthiwong and Rojsitthisak 2011). Alginate has many desirable properties, such as biodegradability, biocompatibility, non-toxicity, gelation and mucoadhesion (Lapasin and Pricl 1995; Phillips et al. 1990; Tønnesen and Karlsen 2002), and is hemocompatible and does not accumulate in organs due to *in vivo* degradation. Thus, alginate has been used in numerous biomedical applications, and especially in drug delivery systems (Motwani et al. 2008; Tønnesen and Karlsen 2002).

3.2. Chitosan

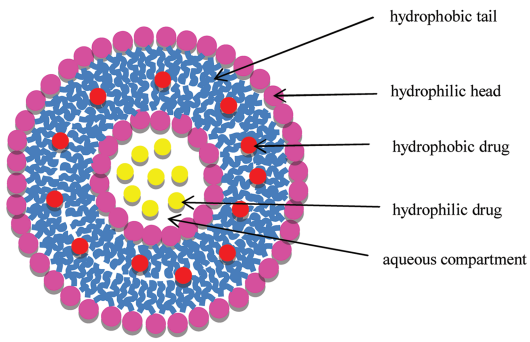
Chitosan is a cationic polysaccharide consisting of copolymers of D-glucosamine and *N*-acetyl-D-glucosamine units linked by β-(1–4) glycosidic linkages (Fig. 3) (Lertsutthiwong and Rojsitthisak 2011). The biocompatibility, non-toxicity, gelation, biodegradability, and membrane permeability of chitosan makes it a polymer of choice for medical and pharmaceutical applications (Agnihotri et al. 2004; De and Robinson 2003; Douglas and Tabrizian 2005; Jayakumar et al. 2010, Sarmento et al. 2007). Chitosan can be degraded by human enzymes, especially lysozyme, and can be fabricated into various forms, such as films, beads, microparticles and nanoparticles (George and Abraham 2006).

3.3. Alginate and chitosan in drug delivery systems

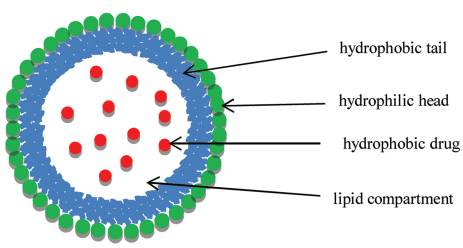
Alginate and chitosan are of interest as biomaterials for use in drug delivery systems due to their versatile properties including non-immunogenicity (Richardson et al. 2001). Chitosan can interact with negatively charged polymers such as alginate and form hydrogels with desirable features for drug encapsulation and drug delivery (Das et al. 2010; Douglas and Tabrizian 2005; Lertsutthiwong and Rojsitthisak 2011; Li et al. 2008). Alginate-



Niosome

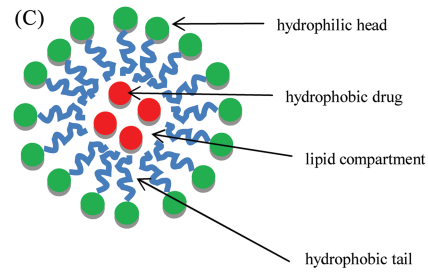


Liposome

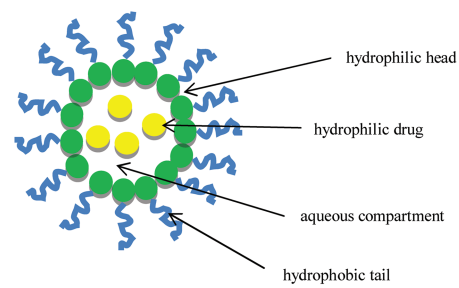


Solid lipid nanoparticle

Fig. 1: *Continued*



O/W nanoemulsion



W/O nanoemulsion

Fig. 1: *Continued*

chitosan nanoparticles are formed by ionotropic gelation based on an interaction between carboxylate groups of alginate and amino groups of chitosan. Alginate-chitosan nanoparticles protect the encapsulated drug from enzymatic degradation, deliver the drug to the target organ, and permit controlled release of the drug (Li et al. 2008). Many studies have focused on the preparation of alginate-chitosan nanoparticles containing various anticancer drugs and targeting different types of cancer cells (Das et al. 2010; Parveen et al. 2010), as shown in Table 2. In addition, chitosan and alginate can be fabricated in the form of microparticles and multilayers.

3.4. Characteristics of alginate-chitosan nanoparticles for drug delivery systems

3.4.1. Particle size

Particle size and size distribution of nanoparticles are used for process control or delivery of a drug to target cells, and these characteristics affect the stability, drug loading and drug release of the nanoparticles (Singh and Lillard Jr 2009). Typically, the cell uptake efficiency of nanoparticles is higher than that of microparticles due to their small size and good mobility. Nanoparticles are also suitable for both cellular and intracellular targeting. Das et al. (2010) demonstrated that curcumin-loaded alginate/chitosan/PF127 nanoparticles with a mean size of 100 nm could be internalized in the HeLa cell line (cervical cancer). Parveen et al. (2010) found that the drug release profile of carboplatin-loaded alginate-chitosan nanoparticles in a retinoblastoma cell line included fast release of 25% of the drug in the first 24 h, followed by sustained release. These results demonstrate that anticancer drug-loaded alginate-chitosan nanoparticles can sustain the antiproliferative activity of a drug in a dose- and time-dependent manner.

3.4.2. Surface properties

Reduction of opsonization and prolongation of the circulation time increase the efficiency of drug targeting and can be achieved by formulation of nanoparticles with a hydrophilic and biodegradable copolymer or coating of the nanoparticles with hydrophilic polymers and surfactants, such as polyoxamers, polyethylene glycol (PEG), polysorbate 80 (Tween 80®) and polyethylene oxide (Singh and Lillard Jr 2009). Alginate-chitosan nanoparticles are biodegradable copolymers with hydrophilic characteristics that reduce opsonization and prolong the circulation time *in vivo*. Das et al. (2010) showed that curcumin-loaded alginate/chitosan/PF127 nanoparticles were effective for passive targeting of cancer cells and prolonged the period the drug spends in the circulation.

The zeta potential or surface charge determines the stability of nanoparticles in suspension due to the electrostatic potential between the particles (Lertsutthiwong et al. 2008, 2009). A zeta potential higher than ± 30 mV produces a stable suspension due to the surface charges preventing aggregation of the particles (Singh and Lillard Jr 2009). For example, the zeta potential of carboplatin-loaded alginate-chitosan nanoparticles prepared by Parveen et al. (2010) was about + 36 mV and no aggregation was observed. Similar results were reported by Coppi and Iannucelli (2009) for tamoxifen-loaded alginate-chitosan nanoparticles, which also had a zeta potential of about + 36 mV. The positive

Table 1: Examples of polymeric nanoparticles for cancer treatment

Polymer	Preparation technique	Anticancer drug	Outcome of study	Reference
Gelatin	Desolvation	Paclitaxel	Rapid drug release from gelatin nanoparticles was observed (~90% released at 37 °C after 2 h)	Lu et al. (2004)
Poly(butylcyanoacrylate) (PBC)	Anionic polymerisation	Doxorubicin	Concentration of doxorubicin in the brain after systemic administration increased about 60-fold by incorporating doxorubicin into PBC nanoparticles coated with polysorbate 80.	Gulyaev et al. (1999)
Poly(lactic-co-glycolic acid) (PLGA)	Nanoprecipitation	9-Nitrocamptothecin	Nanoparticles improved the release profile and sustained release of the drug up to 160 h.	Derakhshandeh et al. (2007)
PLGA	Interfacial deposition	Paclitaxel	Incorporation of paclitaxel in PLGA nanoparticles enhanced the cytotoxicity of paclitaxel compared to a commercial formulation taxol®.	Fonseca et al. (2002)
PLGA	Solvent displacement	Xanthones	Nanoparticles containing xanthones showed good physical stability at 4 °C for 3–4 months.	Teixeira et al. (2005)
PLGA	Interfacial Deposition	Rose bengal	PLGA nanoparticles improved the half-life of rose bengal in the blood stream compared to free drug solution.	Redhead et al. (2001)
PLGA	Double emulsion and solvent evaporation	Triptorelin	Encapsulation efficiency varied from 4% to 83% depending on the interaction between the drug molecule and copolymer. The release profile of triptorelin from PLGA nanoparticles did not show burst effects.	Nicoli et al. (2001)
PLGA	Solvent evaporation	Dexamethasone	Highest drug loading was obtained using 10 mg of dexamethasone and 100 mg PLGA (75:25) in a mixture of acetone-dichloromethane 1:1 (v/v) and complete drug release was observed after 4 h incubation at 37 °C.	Gómez-Gaete et al. (2007)

surface charge may also improve the association of the alginate-chitosan nanoparticles with the cell surface and increase uptake into cancer cells, since most epithelial cells carry a negative charge (Coppi and Iannuccelli 2009).

3.4.3. Drug loading and encapsulation efficiency

A successful NPDDS requires a high drug loading capacity, which can be achieved by reduction of the quantity of matrix material. High drug loading can be accomplished during preparation of nanoparticles and incubation after formation of the particles. Drug loading and entrapment efficiency depend on the method of preparation and the physicochemical properties of the drug (Agnihotri et al. 2004; Kumari et al. 2010; Mohanraj and Chen 2006). Agnihotri et al. (2004) showed that chitosan-based particles could be used for encapsulation of both water-soluble and water-insoluble drugs, with an encapsulation efficiency of 99% for cisplatin. Coppi and Iannuccelli (2009) found that a 92% drug loading capacity could be achieved using alginate-chitosan nanoparticles. These results show that alginate and chitosan are good choices as polymers for formulation of nanoparticles for inclusion of anticancer drugs.

3.4.4. Drug release from alginate-chitosan nanoparticles

Many techniques are used for determination of drug release profiles, including dialysis bag diffusion, reverse dialysis bag

Table 1: *Continued*

Polymer	Preparation technique	Anticancer drug	Outcome of study	Reference
Chitosan	Microemulsion	Doxorubicin conjugated with dextran	Nanoparticles enhanced permeability and the retention effect of doxorubicin.	
			Nanoparticles improved therapeutic efficacy and minimized side effects of doxorubicin.	Mitra et al. (2001)
Chitosan	Novel emulsion droplet coalescence	Gadopentetic acid	Gadopentetic acid was successfully incorporated into chitosan nanoparticles by a novel emulsion droplet coalescence method..	Tokumitsu et al. (1999)
			Chitosan nanoparticles prolonged retention of gadopentetic acid in the tumor tissue.	Shikata et al. (2002)
			Chitosan nanoparticles had high affinity to tumor cells, resulting in greater accumulation in the cells	
Alginate and chitosan	Ionotropic pre-gelation followed by polycationic cross-linking	Curcumin	Composite nanoparticles can deliver a hydrophobic drug like curcumin to cancer cells.	Das et al. (2010)
			Nanoparticles made with this method have a suitable size, encapsulation efficiency and release profile.	
Alginate and chitosan	Pre-gel preparation	Antisense oligonu-cleotide	Alginate/chitosan nanoparticles have the potential to be used as antisense oligonucleotide delivery vectors. The optimal formulation for preparation of alginate/chitosan nanoparticles was alginate/chitosan ratio of 1, $CaCl_2$/alginate ratio of 0.2% and chitosan amine groups/antisense phosphate groups (N/P) ratio of 5 at pH 5.3.	Gazori et al. (2009)
Alginate and chitosan	Ionotropic gelation	Carboplatin	Nanoparticles showed high drug loading, fast release of the drug during the first 24 h, followed by sustained release. High cellular drug uptake and sustained intracellular drug retention were observed.	Parveen et al. (2010)

diffusion, agitation by ultracentrifugation or centrifugation, side-by-side diffusion cells with a membrane, and ultrafiltration (Ringe et al. 2004). Among these techniques, the dialysis method is preferred. Effective release of the encapsulated drug from the alginate-chitosan nanoparticles after internalization into the cancer cells is a key issue in making effective nanoparticles for drug delivery and targeting. Singh and Lillard Jr (2009) demonstrated that burst release of carboplatin from alginate-chitosan nanoparticles occurred within 12 h and that later sustained release also occurred. In a study of curcumin-loaded alginate/chitosan/PF127 nanoparticles, Das et al. (2010) showed that about 36% of the curcumin was released in the first 12 h and 51% in 24 h, followed by sustained release until 72 h, after which the release rate dropped and a

Table 2: Forms of chitosan and alginate for encapsulation of anticancer drugs and targeting of different types of cancer cells

Form	Polymer	Anticancer drug	Targeted organ/cancer cell	Reference
Nanoparticles	Chitosan	Doxorubicin	Murine macrophage cell line	Mitra et al. (2001)
	Chitosan	Gadolinium	L929 mouse fibroblast cells, B16F10 melanoma cells SCC-VII squamous cell carcinoma	Shikata et al. (2002)
	Chitosan	5-Flurouracil (5FU)	HT-29 colon cancer cell line	Jain and Jain (2008)
	Alginate/chitosan	5-Flurouracil (5FU)	Epithelial cells of eyes (ocular delivery)	Nagarwal et al. (2012)
	Alginate/chitosan	Curcumin	Human cervical cancer cell line (HeLa)	Das et al. (2010)
	Alginate/chitosan	Antisense oligonucleotides	T47D breast cancer cell line	Gazori et al. (2010)
	Alginate/chitosan	Carboplatin	Retinoblastoma cell line	Parveen et al. (2010)
Microparticles	Alginate/chitosan	Tamoxifen	Gut-associated lymphoid tissue	Coppi and Iannuccelli (2009)
	Alginate/chitosan	Interleukin-2	Human lung squamous carcinoma SQ-5, and AOI cells Human lung adenocarcinoma A549 cells	Liu et al. (1997)
	Alginate/chitosan	5-Aminosalicylic acid	Colon tissue	Mladenovska et al. (2007)
Multilayers	Alginate/chitosan	Doxorubicin	-	Peng et al. (2010)
	Alginate/chitosan	Doxorubicin	HepG2 tumor cell line	Zhao et al. (2007)

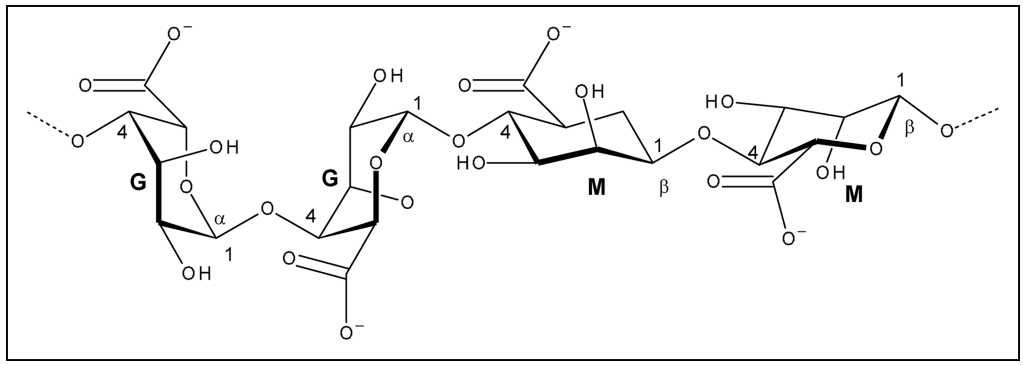


Fig. 2: Chemical structure of alginate. G: guluronic acid; M: mannuronic acid.

total of 75% was released in 96 h. Various mathematic models are used to study the drug release mechanism, including use of the regression coefficient (R^2) to indicate the level of release (Shoaib et al. 2006). For example, the release of curcumin from alginate/chitosan/PF127 nanoparticles had a high R^2 value (0.9421), which indicates good release and a prolonged period in the circulation (Das et al. 2010). Due to the rate of drug release, alginate-chitosan nanoparticles are good candidates as anticancer drug nanocarriers for targeting of cancer cells.

3.4.5. Cytotoxicity and cellular uptake of alginate-chitosan nanoparticles

Alginate-chitosan nanoparticles can be used to deliver anticancer drugs to target cancer cells and induce cell death. Parveen et al. (2010) found that carboplatin-loaded alginate-chitosan nanoparticles affected the viability of the human retinoblastoma cell line Y79. The antiproliferative activity and apoptosis rate of cancer cells induced by the carboplatin-loaded alginate-chitosan nanoparticles were greater than those with the native (unencapsulated) drug. This activity increased with incubation time when the cancer cells were treated with a low dose of carboplatin loaded into the alginate-chitosan nanoparticles. Moreover, the IC_{50} of the carboplatin-loaded alginate-chitosan nanoparticles was lower than that of native carboplatin (Parveen et al. 2010). Curcumin-loaded alginate-chitosan-PF127 nanoparticles were successfully delivered into HeLa cells based on the green fluorescence in fluorescent microscopy images of these cells after treatment with the nanoparticles (Das et al. 2010).

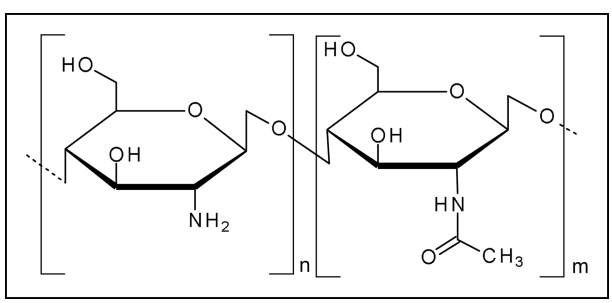


Fig. 3: Chemical structure of chitosan.

4. Conclusion

Anticancer drugs are typically toxic and harmful to healthy cells, which is a major disadvantage of chemotherapy. To overcome this problem, drug delivery systems are required as novel therapy. Nanoparticles are preferable for delivery of anticancer drugs due to their ease of intracellular uptake and the increased efficacy of therapy. The smaller size of nanoparticles allows penetration across blood capillaries and uptake into cancer cells with high efficiency. Such a nanoparticulate drug delivery system can be used to deliver drugs to target organs and improve important parameters such as oral bioavailability, stability of

chemotherapy agents against enzymatic degradation, reduction of drug toxicity, and therapeutic efficacy. Biodegradable polymers such as alginate and chitosan are useful for preparation of nanoparticles and are a good choice as anticancer drug nanocarriers in drug delivery systems that may replace conventional cancer chemotherapy.

Acknowledgments: The authors express their thanks to the Ph.D. Programm in Nanoscience and Technology, (International) Graduate School, Chulalongkorn University for a Scholarship and the Ratchadaphiseksomphot Endowment Fund of Chulalongkorn University (RES560530014-AM) for providing research funds. We also thank Dr. Ian S. Haworth (University of Southern California) for his comments and suggestions on the manuscript.

References

Agnihotri SA, Mallikarjuna NN, Aminabhavi TM (2004) Recent advances on chitosan-based micro- and nanoparticles in drug delivery. J Control Release 100: 5–28.

American Cancer Society (2011) Cancer Facts & Figures 2011. Atlanta: American Cancer Society.

Anand P, Kunnumakkara AB, Sundaram C, Harikumar KB, Tharakan ST, Lai OS, Sung B, Aggarwal BB (2008) Cancer is a preventable disease that requires major lifestyle changes. Pharm Res 25: 2097–2116.

Bawa R (2008) Nanoparticle-based therapeutics in humans: a survey. Nanotechnol Law Business 5: 135–155.

Chellat F, Merhi Y, Moreau A, Yahia L (2005) Therapeutic potential of nanoparticulate systems for macrophage targeting. Biomaterials 26: 7260–7275.

Colvin VL (2003) The potential environmental impact of engineered nanomaterials. Nat Biotechnol 21: 1166–1170.

Coppi G, Iannuccelli V (2009) Alginate/chitosan microparticles for tamoxifen delivery to the lymphatic system. Int J Pharm 367: 127–132.

Das RK, Kasoju N, Bora U (2010) Encapsulation of curcumin in alginate-chitosan-pluronic composite nanoparticles for delivery to cancer cells. Nanomedicine 6: 153–160.

Davis ME (2006) Nanoparticles for systemic medicines and imaging agents. Nanotechnol Law Business 3: 255–261.

De S, Robinson D (2003) Polymer relationships during preparation of chitosan-alginate and poly-l-lysine-alginate nanospheres. J Control Release 89: 101–112.

Decker-Baumann C, Buhl K, Frohmüller S, Herbay AV, Dueck M, Schlag PM (1999) Reduction of chemotherapy-induced Side-effects by parenteral glutamine supplementation in patients with metastatic colorectal cancer. Eur J Cancer 35: 202–207.

Derakhshandeh K, Erfan M, Dadashzadeh S (2007) Encapsulation of 9-nitrocamptothecin, a novel anticancer drug, in biodegradable nanoparticles: factorial design, characterization and release kinetics. Eur J Pharm Biopharm 66: 34–41.

Derfus AM, Chan WCW, Bhatia SN (2004) Probing the cytotoxicity of semiconductor quantum dots. Nano Lett 4:11–18.

Douglas KL, Tabrizian M (2005) Effect of experimental parameters on the formation of alginate-chitosan nanoparticles and evaluation of their potential application as DNA carrier. J Biomater Sci Polym Ed 16: 43–56.

Fonseca C, Simões S, Gaspar R (2002) Paclitaxel-loaded PLGA nanoparticles: preparation, physicochemical characterization and *in vitro* anti-tumoral activity. J Control Release 83: 273–286.

Gazori T, Khoshayand MR, Azizi E, Yazdizade P, Nomani A, Haririan I (2009) Evaluation of alginate/chitosan nanoparticles as antisense delivery vector: Formulation, optimization and in vitro characterization. Carbohydr Polym 77: 599–606.

Gazori T, Haririan I, Fouladdel S, Namazi A, Nomani A, Azizi E (2010) Inhibition of EGFR expression with chitosan/alginate nanoparticles encapsulating antisense oligonucleotides in T47D cell line using RT-PCR and immunocytochemistry. Carbohydr Polym 80: 1042–1047.

Gelperina S, Kisich K, Iseman MD, Heifets L (2005) The potential advantages of nanoparticle drug delivery systems in chemotherapy of tuberculosis. Am J Respir Crit Care Med 172: 1487–1490.

George M, Abraham TE (2006) Polyionic hydrocolloids for the intestinal delivery of protein drugs: Alginate and chitosan- a review. J Control Release 114: 1–14.

Gómez-Gaete C, Tsapis N, Besnard M, Bochot A, Fattal E (2007) Encapsulation of dexamethasone into biodegradable polymeric nanoparticles. Int J Pharm 331: 153–159.

Gulyaev AE, Gelperina SE, Skidan IN, Antropov AS, Kivman GY, Kreuter J (1999) Significant transport of doxorubicin into the brain with polysorbate 80-coated nanoparticles. Pharm Res 16: 1564–1569.

Haley B, Frenkel E (2008) Nanoparticles for drug delivery in cancer treatment. Urol Oncol 26: 57–64.

Jain A, Jain SK (2008) *In vitro* and cell uptake studies for targeting of ligand anchored nanoparticles for colon tumors. Eur J Pharm Sci 35: 404–416.

Jayakumar R, Menon D, Manzoor K, Nair SV, Tamura H (2010) Biomedical applications of chitin and chitosan based nanomaterials-A short review. Carbohydr Polym 82: 227–232.

Juillerat-Jeanneret L (2008) The targeted delivery of cancer drugs across the blood-brain barrier: chemical modifications of drugs or drug-nanoparticles? Drug Discov Today 13: 1099–1106.

Kukowska-Latallo JF, Candido KA, Cao Z, Nigavekar SS, Majoros IJ, Thomas TP, Balogh LP, Khan MK, Baker JR Jr (2005) Nanoparticle targeting of anticancer drug improves therapeutic response in animal model of human epithelial cancer. Cancer Res 65: 5317–5324.

Kumar MNVR (2000) Nano and microparticles as controlled drug delivery devices. J Pharm Pharmaceut Sci 3: 234–258.

Kumari A, Yadav SK, Yadav SC (2010) Biodegradable polymeric nanoparticles based drug delivery systems. Colloids Surf. B: Biointerfaces 75: 1–18.

Lapasin R, Pricl, S (1995) Rheology of industrial polysaccharides: Theory and applications. UK: Blackie Academic and Professional.

Lertsutthiwong P, Noomun K, Jongaroonngamsang N, Rojsitthisak P, Nimmannit U (2008) Preparation of alginate nanocapsules containing turmeric oil. Carbohydr Polym 74: 209–214.

Lertsutthiwong P, Rojsitthisak P, Nimmannit U (2009) Preparation of turmeric oil-loaded chitosan-alginate biopolymeric nanocapsules. Mater Sci Eng C 29: 856–860.

Lertsutthiwong P, Rojsitthisak P (2011) Chitosan-alginate nanocapsules for encapsulation of turmeric oil. Pharmazie 66: 911–915.

Li P, Dai YN, Zhang JP, Wang AQ, Wei Q (2008) Chitosan-alginate nanoparticles as a novel drug delivery system for nifedipine. Int J Biomed Sci 4: 221–228.

Liu LS, Liu SQ, Ng SY, Froix M, Ohno T, Heller J (1997) Controlled release of interleukin-2 for tumour immunotherapy using alginate /chitosan porous microspheres. J Control Release 43: 65–74.

Lu Z, Yeh TK, Tsai M, Au JLS, Wientjes MG (2004) Paclitaxel-loaded gelatin nanoparticles for intravesical bladder cancer therapy. Clin Cancer Res 10: 7677–7684.

Malesu VK, Sahoo D, Nayak PL (2011) Chitosan-sodium alginate nanocomposites blended with cloisite 30B as a novel drug delivery system for anticancer drug curcumin. Int J Appl Biol Pharm Technol 2: 402–411.

Mitra S, Gaur U, Ghosh PC, Maitra AN (2001) Tumor targeted delivery of encapsulated dextran-doxorubicin conjugate using chitosan nanoparticles as carrier. J Control Release 74: 317–323.

Mittelberg KN, Tucker RD, Loening SA, Moseley PL (1996) Effect of radiation and hyperthermia on prostate tumor cells with induced thermal tolerance and the correlation with HSP70 accumulation. Urol Oncol 2: 146–151.

Mladenovska K, Raicki RS, Janevik EI, Ristoski T, Pavlova MJ, Kavrakovski Z, Dodov MG, Goracinova K (2007) Colon-specific delivery of 5-aminosalicylic acid from chitosan-Ca-alginate microparticles. Int J Pharm 342: 124–136.

Mohanraj VJ, Chen Y (2006) Nanoparticles-A review. Trop J Pharm Res 5: 561–573.

Motwani SK, Chopra S, Talegaonkar S, Kohli K, Ahmad FJ, Khar RP (2008) Chitosan-sodium alginate nanoparticles as submicroscopic reservoirs for ocular delivery: Formulation, optimization and in vitro characterization. Eur J Pharm Biopharm 68: 513–525.

Nagarwal RC, Kumar R, Pandit JK (2012) Chitosan coated sodium alginate-chitosan nanoparticles loaded with 5-FU for ocular delivery: *In vitro* characterization and *in vivo* study in rabbit eye. Eur J Pharm Sci 47: 678–685.

Nicoli S, Santi P, Couvreur P, Couarraze G, Colombo P, Fattal E (2001) Design of triptorelin loaded nanospheres for transdermal iontophoretic administration. Int J Pharm 214: 31–35.

Oberdörster G, Oberdörster E, Oberdörster J (2005) Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ Health Perspect 113: 823–839.

Pandey R, Khuller GK (2006) Oral nanoparticle-based antituberculosis drug delivery to the brain in an experimental model. J Antimicrob Chemother 57: 1146–1152.

Parveen S, Mitra M, Krishnakumar S, Sahoo SK (2010) Enhanced antiproliferative activity of carboplatin-loaded chitosan-alginate nanoparticles in a retinoblastoma cell line. Acta Biomater 6: 3120–3131.

Peng C, Zhao Q, Gao C (2010) Sustained delivery of doxorubicin by porous $CaCo_3$ and chitosan/alginate multilayers-coated $CaCo_3$ microparticles. Colloids Surf A: Physicochem Eng Aspects 353: 132–139.

Phillips GO, Williams PA, Wedlock DJ (1990) Gums and stabilizers for the food industry 5: Applications of alginates. New York: IRL Press at Oxford University Press.

Redhead HM, Davis SS, Illum L (2001) Drug delivery in poly(lactide-co-glycolide) nanoparticles surface modified with poloxamer 407 and poloxamine 908: *in vitro* characterisation and *in vivo* evaluation. J Control Release 70: 353–363.

Richardson TP, Murphy WL, Mooney DJ (2001) Polymeric delivery of proteins and plasmid DNA for tissue engineering and gene therapy. Crit Rev Eukaryot Gene Expr 11: 47–58.

Ringe K, Walz CM, Sabel BA (2004) Nanoparticle drug delivery to the brain. In: Nalwa HS (ed.) Encyclopedia of Nanoscience and Nanotechnology 7: 91–104.

Sarmento B, Ferreira DC, Jorgensen L, van de Weert M (2007) Probing insulin's secondary structure after entrapment into alginate/chitosan nanoparticles. Eur J Pharm Biopharm 65: 10–17.

Shikata F, Tokumitsu H, Ichikawa H, Fukumori Y (2002) *In vitro* cellular accumulation of gadolinium incorporated into chitosan nanoparticles designed for neutron-capture therapy of cancer. Eur J Pharm Biopharm 53: 57–63.

Shoaib MH, Tazeen J, Merchant H, Yousuf RI (2006) Evaluation of drug release kinetics from ibuprofen matrix tablets using HPMC. Pak J Pharm Sci 19: 119–124.

Shukla RK, Tiwari A (2012) Carbohydrate polymers: Applications and recent advances in delivering drugs to the colon. Carbohydr Polym 88: 399–416.

Singh R, Lillard Jr JW (2009) Nanoparticle-based targeted drug delivery. Exp Mol Pathol 86: 215–223.

Subramani K (2009) NPDDS for cancer treatment: targeting nanoparticles, a novel approach. In: Pathak Y, Thassu D (eds): Drug Delivery Nanoparticles Formulation and Characterization. Drugs and the Pharmaceutical Sciences vol. 191, pp. 57–68.

Teixeira M, Alonso MJ, Pinto MMM, Barbosa CM (2005) Development and characterization of PLGA nanospheres and nanocapsules containing xanthone and 3-methoxyxanthone. Eur J Pharm Biopharm 59: 491–500.

Tokumitsu H, Ichikawa H, Fukumori Y (1999) Chitosan-gadopentetic acid complex nanoparticles for gadolinium neutron-capture therapy of cancer: preparation by novel emulsiondroplet coalescence technique and characterization. Pharm Res 16: 1830–1835.

Tønnesen HH, Karlsen J (2002) Alginate in drug delivery systems. Drug Dev Ind Pharm 28: 621–630.

Zhao Q, Han B, Wang Z, Gao C, Peng C, Shen J (2007) Hollow chitosan-alginate multilayer microcapsules as drug delivery vehicle: doxorubicin loading and *in vitro* and *in vivo* studies. Nanomedicine 3: 63–74.