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Potential of Chitosan and its derivatives for controlled drug release applications – A review

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ABSTRACT

Recent research on the drug delivery systems exhibited tremendous improvements for several short life drugs which disappear in few minutes in harsh conditions of the Gastrointestinal tract (GIT). After years of investigations, the current drug delivery system has been improved with new advanced materials with less toxicity and better therapeutic efficiency. In this regard, new formulations consisting of drugs encapsulated with natural biodegradable copolymer, Chitosan, in the form of nanoparticles have been studied, which in turn improved the release profile of drugs. In this review, the Chitosan and its physiochemical properties, nanoparticles and their drug release mechanism and effects of modification of various drugs (anti–cancer, anti–inflammatory, anti–-diabetes, anti–infectious drugs etc) with Chitosan and co–materials on their release profiles are briefly reviewed. These biodegradable polymeric nanoparticles improved the in vitro release profile of drugs and provided a way forward for further improvement of the current and conventional drug delivery systems.

1. Introduction

The controlled and sustained release of drugs has become a promising research domain. Scientists, researchers and drug specialists are working hard for the development of such drug delivery system (DDS) which could provide an appropriate drug concentration to meet the therapeutic needs. The uncontrolled drug release initially results in highly toxic concentration and afterward in an ineffective concentration of drug which in turn causes severe health issues [1,2]. This uneven behaviour is due to the nature of drugs and the route of administration. Some of the drugs have a short life and rapidly consumed by the system resulting in lower plasma level. Therefore, more amount of the drug is required to maintain the plasma level which ultimately increases the dosage and hence resulting in more discomfort to patients. Mitomycin C (MMC) is an anti–cancer drug which has a short life and serious side effects (SSE) including the destruction of bone marrow and other tissues [3]. Docetaxel (DCT) possesses the similar shortcomings with the disadvantage of poor aqueous solubility and non–targeting delivery [4]. Similarly, the low aqueous solubility of Paclitaxel (PTX) is compensated by the addition of Cremophor while compromising the side effects (labored breathing, hypersensitivity, neurotoxicity and hypotension). To overcome these shortcomings and to provide a sustained release of short life drugs, a multi–controlled DDS is essential [5,6].

Currently, the drug administration is being carried out preferably by both invasive and non–invasive routes [7], where the parenteral, intravenous, intracellular and intramuscular routes belong to the invasive route of administration [8–12]. Some of the drugs have limitations of administration through invasive routes because of their side effects which might lead to the death of the patient. The human African trypanosomiasis (HAT) is one of the severe infectious diseases where the drug doses are restricted to administer through the invasive routes [13]. In some cases, the doses of drugs such as insulin through the invasive routes do not fulfill the therapeutic requirements and an excess amount of drug is needed. The repeated injection causes serious side effects (SSE) such as lipoatrophy/lipohypertrophy, allergic reactions, infections and peripheral hyperinsulinemia and abnormal proliferation of muscle cells [14]. The overview of these shortcomings clearly indicates the need for new strategies, which might avoid the repetition of effective doses and hence to minimize the toxic effects.

On the other hand, many anti–infection, anti–diabetic and anti–cancer drugs are administered by non–invasive routes including oral, nasal and buccal routes [15]. Oral chemotherapy is preferable due to ease of drug intake by the patients, but this route also faces several challenges and limitations. The major barrier of this route is

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Nomenclature

BDP	Betamethasone dipropionate
BHEM	N, N–bis(2–hydroxyethyl)–N–methyl–N–(2–cholesteryloxycarbonyl aminoethyl)
BP	Betamethasone phosphate
BSA	Bovine Serum Albumin
CMCS	Carboxymethyl Chitosan
CP	Chloroquine Phosphate
CPT	Camptothecins
CyA	Cyclosporin A
Da	Dalton
DEM	Dexamethasone
DGL	Dendritic poly–L– lysine
DOX	Doxorubicin
DCT	Docetaxel
DPPC	1, 2–Dipalmitoyl–sn–glycero–3–phosphocholine
EMA	European medicine agencies
FA	Floate; FDA Food and Drug Administration
Gal	Galactosamine; GC Glycol Chitosan
Gel	Gelatin
GIT	Gastro Intestinal Tract; GMO Glyceryl Monooleate
GNPs	Gold Nanoparticles
HAT	Human African Trypanosomiasis
HGNs	Gold nanoshells
HOs	Hydrotropic Oligomers
IBU	Ibuprofen
MAMA	9–(methylaminomethyl)anthracene
MEC	Minimum effective concentration
MM	Molecular mass
MMC	Mitomycin C
MT	Montmorillonite
MPEG	Methoxy poly(ethylene glycol
NIR	Near infrared resonant
NPs	Nanoparticles
Nm	Nanometer
PBS	Phosphate buffer solution
PAMPS	Poly(2–acry1amido–2–methylpropanesulfonic acid)
PCL	Poly–alkyl–acyanoacrylate
PD	Polydopamine
PEG	Polyethylene glycol
PEPLA	Lipid–Poly lactic acid
PLA	Poly lactic acid
PLGA	Poly lactic–co–glycolic acid
PTX	Paclitaxel
siRNA	small interfering RNA
SPC	Soybean phosphatidylcholine
Sty	Styrene
T	Tween
TEA	Triethylamine
TMX	Tamoxifen
TPGS	d–α–tocopheryl polyethylene glycol 1000 succinate
Tg	Glass Transition temperature
VF	Venlafaxine hydrochloride

gastrointestinal tract (GIT) which contains an extremely acidic pH medium. The exposure of pH–sensitive drug into the GIT results in sudden degradation of drug and its concentration fell down below the minimum effective concentration (MEC) value of the drug and hence resulting in ineffectiveness [16–18]. The sparingly water– soluble drugs belong to this category as their administration is ineffective through the oral route. The release profiles of several drugs have clearly shown that they are lacking controlled release and need some suitable carriers which may control the release rate and mechanism of the respective drug. These carrier materials could also be useful to enhance the drug absorption in blood as a replacement of the side effect transmitting surfactants, fatty acids and bile salts. In this context, two approaches were highlighted and are under investigation. The first approach is the development of new drugs which may have better characteristics than the existing conventional drugs, while the second approach is the optimization of conventional drug formulations by using suitable carrier materials [19]. Several carrier materials, namely inorganic, magnetic and polymeric nanoparticles (PNPs) have been explored for drug delivery applications [20,21]. Based on the above–mentioned aspects, the PNPs are considered more advanced than other simple NPs for the targeted drug delivery systems (TDDS). The PNPs are classified into two groups; one as biodegradable synthetic polymers (Poly lactic–co–glycolic acid (PLGA), Poly lactic acid (PLA) and Poly–alkyl–cyanoacrylate (PCL) etc) while the other group is known as natural biodegradable polymers (Chitosan (CS), Alginate, Hyaluronic acid, Dextran, Collagen, Albumin, Elastin and Gelatin etc) [22–24]. Chitosan is one of the natural biopolymers which is widely investigated for the drug modification applications. This copolymer alone or with other materials has imparted its targeted characteristics to the drug formulations, which resulted in controlled DDS/prolonged release system. These surface modifications improved the overall efficiency of drugs by controlling the degradation and release mechanism. The aim of this paper is to provide a brief review on Chitosan (CS), its physiochemical properties and application in the field of drug release with a deep understanding of CS NPs and their role in drug release.

1.1. Chitosan and its chemistry

Chitosan is a stable, safe, biocompatible, bioactive and biodegradable polysaccharide consists of glucosamine and N–acetylglucosamine which is being prepared by deacetylation of naturally occurring chitin [25–31]. This deacetylation occurs at 100–160 °C by using 40–50% aqueous alkaline solution resulting in deacetylation degree up to 0.95. Chitosan chemistry reveals that it is immiscible in water due to non-protonation at neutral and alkaline pH conditions [32]. Since the permeation mechanism is based on positive charges of CS and these charges can enhance the permeation property and structural rearrangement, resulting in tight junction protein association [33]. Due to the cationic nature, it possesses mucoadhesive properties which are useful for the improvement of oral bioavailability of drugs [34–36]. Though these properties are weaker than many other anionic polymers but can be improved further by its modification [37]. It has an ability of self–branching, which is an important property for the gene transfer applications and can enhance the gene expression with a better safety profile [38]. The structure of CS can be seen in Fig. 1. The presence of free amino groups in CS plays an important role in crosslinking with other anions enabling it suitable for various applications such as food

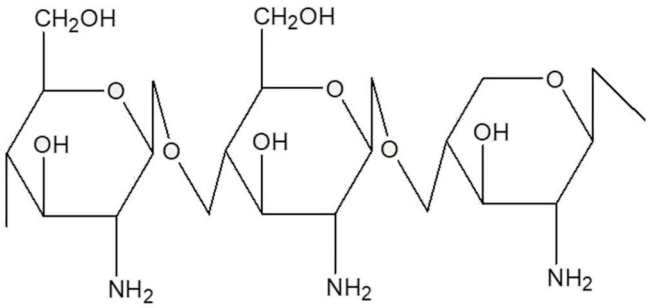

Fig. 1. Structure of Chitosan.

Table 1
Characteristics of various drugs encapsulated with chitosan and its derivatives.

Drug loaded with Chitosan and Chitosan derivatives NPs.	Drugs/Release Mechanism	Method of preparation for drug loaded NPs	Type of Drug Release		In Vitro Conditions
			Burst Release (%) and Time	Slow Release (%) and Time	pH = /Temperature (°C)
Chitosan– Carboxymethyl Chitosan [78] Chitosan (MM = 10 kDa and DDA = 89%)	DOX/NA	Ionic Gelation	45% in 4 h 35% in 4 h 50% in 4 h	44.99% after 60 h 38.60 after 60 h 79.36% after 60 h	pH = 1.20 at 37 °C pH = 6.0 at 37 °C pH = 7.0 at 37 °C
Chitosan (MM = 7–200 kDa) [81]	Ammonium glycyrrhizinate/Diffusion and degradation	Ionic Gelation	20% in 1 h and 35% in 10 h	53% in 3 days	PBS with pH = 7.40 at 37 °C
Chitosan–Katira gum [84]	Glycyrrhizic acid/Diffusion and erosion	Ionic Gelation	≤5.0% in 60 min	90.71% in 12 h	PBS with pH = 7.40 at 37 °C
Chitosan (MM = 550 kDa and DDA = 75% and 90%) [113]	Fluorouracil (5-FU)/Diffusion	Ionic Gelation	≥55%) in 10 h for 90% DDA ≥75%) in 10 h for 75% DDA	(≥75%) in 100 h for 90% DDA ≥85%) in 100 h for 75% DDA	PBS with pH = 7.0 at 37 °C
Hydrotropic Oligomer–Chitosan [114] Chitosan (MM = 250 kDa and DDA = 82.70%)	Paclitaxel/NA	Dialysis	20–30% in 5 h	80–100% in 160 h	PBS with pH = 7.40 at 37 °C
mPEGylated Chitosan [122] Chitosan (MM = 120 kDa and DD of 75–85%)	Cyclosporin A (CyA)/Diffusion	Emulsification/Solvent Evaporation Method	≥15.0% in 10 h	32.6% in 48 h	0.1 M PBS with pH = 7.40 at 37 °C
Free Cyclosporin A in aqueous solution [122]				4.0% in 52 h	0.1 M PBS with pH = 7.40 at 37 °C
Dimethyl–β–cyclodextrin–Chitosan [123] Chitosan (MM = 100 kDa and DDA = 88.5%	Salazosulfapyridine (SASP)/Diffusion and degradation	Freeze drying method	≥42% in 4 h	≥75% in 24 h	Simulated intestinal fluid with pH = 6.80
Salazosulfapyridine (SASP) [123]			10% in 4 h	15% in 24 h	
Chitosan (MM = 7 kDa) [124]	Podophyllotoxin/Drug desorption and diffusion	Ionic Gelation	15–20% in 15 min	78% in 30 h 60.0% in 30 h 45.0% in 30 h	pH = 3.70 at 37 °C pH = 6.80 at 37 °C pH = 7.40 at 37 °C
N–O–Carboxymethyl Chitosan [127] Chitosan (MM = 100–150 kDa and DD of 80%)	Curcumin/Swelling and diffusion	Ionic Gelation	30% in 12 h	52.0% in 144 h 49.0% in 144 h	pH = 4.50 at 37 °C pH = 7.40 at 37 °C
Chitosan– MCM–41 [130] Chitosan (MM = MMM and DDA = 75–85%)	Curcumin/Diffusion and swelling	Sol Gel	10–22% in 12 h	33.94 ± 2.42% in 32 h) and (42.72 ± 2.29% in 96 h 16.85 ± 1.17% for 32 h and 19.54 ± 1.36% in 72 h	pH = 5.50 at 37 °C 0.1 M PBS with pH = 7.40 at 37 °C
Folate–Chitosan [131] Chitosan (MM = 400 kDa and DDA = 89%)	Curcumin/Desorption and swelling	Stirring and Sonication	10–20% n 3 h	90% in 1 week 75% in 1 week	pH = 5.0 at 37 °C pH = 7.40 at 37 °C
Chitosan (MM = 310 kDa and DDA = 75–85%) [133]	Docetaxel/Diffusion and degradation	NA	≥50% in 2 h ≥12% in 2 h ≥12% in 2 h	100% in 60 h ≥55% in 60 h ≥35% in 60 h	PBS with pH = 1.2 at 37 °C PBS with pH = 5.3 at 37 °C PBS with pH = 7.4 at 37 °C
Clay–Chitosan Chitosan (MM = 310 kDa and DDA = 75–85%) [133]			12–18% in 2 h 3–8% in 2 h 3–6% in 2 h	25–45% in 60 h 18–30% in 60 h 16–32% in 60 h	PBS with pH = 1.2 at 37 °C PBS with pH = 5.3 at 37 °C PBS with pH = 1.2 at 37 °C
CBPA–Chitosan [134] Chitosan (MM = 3 kDa and DDA = ≥ 80%)	Docetaxel/Diffusion and degradation	Stirring in dark	≈40.0% in 12 h ≈32.0% in 12 h ≈14.0% in 12 h	69.20% in 120 h 47.8% in 120 h 22.0% in 120 h	pH = 5.0 at 37 °C pH = 6.0 at 37 °C pH = 7.4 at 37 °C
Carboxymethyl Chitosan [134] Chitosan (Mn = 3 kDa and DDA = ≥ 80%)	Docetaxel/Diffusion and degradation	Stirring in dark	≈46.0% in 10 h ≈30.0% in 10 h ≈10% in 10 h	78.8% in 120 h 54.8% in 120 h 24.2% in 120 h	pH = 5.0 at 37 °C pH = 6.0 at 37 °C pH = 7.4 at 37 °C
Chitosan– Poly(2acry1amido2methylpropanesulfonic acid) [136] Chitosan (MM = 498 kDa and DDA = 80–95%)	Docetaxel/Diffusion	Polyelectrolyte complex	45.3% after 10 h 49.5% after 10 h 53.5% after 10 h 62.4% after 10 h	≥90% in 60 h ≥80% in 60 h ≥70% in 60 h ≥60% in 60 h	PBS with pH = 5.0 PBS with pH = 6.0 PBS with pH = 7.4 PBS with pH = 8.0
Glyceryl Monooleate–Chitosan [137] Chitosan (MM = LMM =)	Paclitaxel/Diffusion	Emulsion/Solvent Evaporation	≥10% in 30 min	≥15% in 4 h	PBS with pH = 7.4 at 37 °C
Glyceryl Monooleate–Chitosan Chitosan (LMM =) [137]	Dexamethasone/Diffusion		≥45% in 30 min	≥55% in 4 h	
Dextran Sulfate–Chitosan [140] Chitosan (MM = 50 kDa and DDA = 15%)	Insulin/Diffusion	NA	5% in 2 h	70% in 6 h	pH = 1.2 and pH = 6.8 respectively at 37 °C

(*continued on next page*)

Table 1 (*continued*)

Drug loaded with Chitosan and Chitosan derivatives NPs.	Drugs/Release Mechanism	Method of preparation for drug loaded NPs	Type of Drug Release		In Vitro Conditions
			Burst Release (%) and Time	Slow Release (%) and Time	pH = /Temperature (°C)
Chitosan SeaCure 123 [148]	Cyclosporin A (CyA)/Diffusion	Ionic Gelation	62% in 15 min	75% in 24 h	37 °C
Chitosan (MM = 40–80 kDa and DDA = 80%) [149]	Docetaxel/Diffusion and dissolution	Ionic Gelation	10–22% in 1 h	70–88% in 25 h	PBS with pH = 7.4 at 37 °C
HTCC [150]	Bovine Serum Albumin/Diffusion,	Ionic Gelation	≈ 45% in 12 h	≈ 57% in 6 days	0.9% (w/v) sodium chloride saline
Chitosan (MM = 210 kDa and DDA = 92%)	polymer degradation and erosion		≈ 30% in 12 h	≈ 35% in 6 days	
Water Soluble Chitosan [151]	Bovine Serum Albumin/Diffusion	Ionic Gelation	30% in 30 min	90% in 24 h	PBS with pH = 7.4 at 37 °C
Chitosan (21 kDa and DDA = 87%)					

and textile industry, wastewater treatment and pharmaceutical applications. The medical perspective of CS includes nontoxicity towards living cells and antimicrobial characteristics against many Gram–positive bacteria (GPB)/Gram–negative bacteria (GNB) with high killing [31]. The level of antimicrobial nature of CS depends on its molecular mass, pH and metal cations activity. Chitosan can absorb toxic metals such as mercury and lead with an additional advantage of nontoxic products on degradation which easily gets accumulated in the human body [39].

The reason for this friendly nature of CS towards the human body is its bioactivity, epithelial permeability, mucoadhesion and biodegradability. Chitosan has been used in various pharmaceutical and medical applications and its potential has already been proved. In DDS, drug modification with CS and its derivatives as a carrier material is more simple and convenient [40]. In this regard, CS is widely used as NPs/MPs, tablets, microcapsules/spheres, hydrogels and beads. Up to date, CS has been used for modification of several proteins, anti–infectious, anti–diabetes and anti–cancer drugs. The characteristics of CS such as non toxicity with additional properties of antibacterial activity, biodegradability, cationic nature to interact with anionic drugs, crosslinking ability and control over swelling and high degree of deacetylation make it more suitable to tailor the release profile of the various drugs. For example, the mechanical strength of the particles increases with increase in crosslinking of CS which means highly crosslinked particles would have less swelling which ultimately results in less inside water penetration and outside drug diffusion. Similarly, control over the degree of deacetylation (DDA) and molecular mass (MM) can control the release profile of drug. Understanding the nature of the properties and optimization of these properties may results in better drug formulations with sustained release rate.

Some studies highlighted that the protein/drug structure and its interaction with the polymer in suspension medium significantly affect the release characteristics [41,42]. Understanding about the interaction mechanism among the CS and various drugs could help in the development of polymer-drug association. The stage and medium are the factors which might change the chemistry of CS and hence influence the interaction mechanism which ultimately changes the characteristic of polymer-drug matrix (mechanical strength) during drug release application. The interaction study between CS and a drug (BSA) indicated that the amine group of the CS and carboxyl group of the BSA play a significant role in the formation of particles. The BSA has high association with CS partly due to the electrostatic interactions, however this association was dependent on pH of CS solution. For the case where, the BSA was dissolved in CS, the highest interaction of BSA with CS was found at pH = 5, where CS remained positively charged (pKa = 6.5) and BSA remained negatively charged (isoelectric point = 4.8). On the other hand, hydrogen bonding and hydrophobic forces are also partly responsible for the enhancement of the BSA entrapment into the CS NPs [43,44]. In comparison to acidic medium CS, the BSA entrapment efficiency was found to be higher when dissolved in TPP (pH = 8.0) and crosslinked with CS. This might be due to the high electronegativity of BSA at this pH which enhanced its interaction with CS. The release profile of BSA showed that the drug loading played a vital role in accelerating the BSA release. Therefore, the optimization of BSA loading could modulate its release rate. The interaction mechanism of CS with other drugs could be different based on their different isoelectric points and their associations might be either due to electrostatic interactions or hydrogen bonding/hydrophobic forces or both [45,46]. Several methods (FTIR, XPS, turbidity measurement, ^{1}H NMR, viscometry, and isothermal titration calorimetry etc) have been investigated to estimate the binding forces between drugs and chitosan/functional groups [47]. The 1H NMR, FTIR spectroscopy and isothermal titration calorimetry have proved the CS interaction with human insulin and benzoic acid. The study of ionic interaction was conducted by using different combinations of benzoic acid and CS [43], again these interactions were pH dependent and lower pH = 3.3 resulted in high entrapment (68–80%)

of benzoic acid into CS NPs as compared with higher pH = 5.0. Different combinations resulted in different peaks forming ortho, para and meta protons. The reason behind the shifting of proton was the dissociation of benzoic acid. For the same drug-polymer system, the FTIR analysis showed that some bands of CS overlapped the insulin bands and hence resulted in wider bands (carbonyl and amine). This widening became more prominent as the insulin concentration increased. The small shifting in fingerprints region showed that some weak interactions also exist between CS and insulin. Microcalorimetry technique estimated the interaction between CS and insulin by measuring the change in enthalpy. The analysis showed a moderate enthalpy change which indicated a partly ionic interaction between CS and insulin. The reason behind this moderate enthalpy change could be importantly due to insulin adsorption on polymer without any binding, structural modification or ionization of polar groups. No doubt, such weak interaction results in burst release of insulin. On the other hand, the rapid release of benzoic acid from CS NPs in phosphatic buffer pH = 7.4 indicated that either CS did not interact or weakly interacted with it. The TPP did not affect the CS other than hydration of polymer and interaction between them is very weak interaction in the presence of benzoic acid [43].

Effects of physiochemical properties of Chitosan on drug release application

Physiochemical properties of the materials play a vital role in the development of a drug delivery system. Therefore, it is necessary to understand these properties prior to their application as a mixed matrix particularly for controlled release of drugs.

Since CS has been recovered from different sources by using different methods, therefore, its properties may vary in terms of purity of the product, MM, DDA, amino and acetamido groups. In general, the MM of CS is between 10,000 and 1 million Dalton (Da), while DDA is found between 50 and 95%. In terms of chemical properties, CS possess a stiff arrangement of D–glucosamine with a high charge density which is counted as one positive charge for each of glucosamine present. It has high nucleophilic nature with an approximate pKa value of 6.50 and able to form hydrogen bonds. The presence of highly reactive groups and hydrophilic nature of CS enable it to form salts with several organic/inorganic acids through hydrogen bonding or crosslinking. Chitosan (CS) is a chelating, viscous, crystalline and polyelectrolyte under acidic conditions [48–50]. The particle size, size distribution, shape, zeta potential, stability, swelling index and release profile are dependent on the purity of polymer, MM, DDA, concentration, initial pH conditions, interaction with substance added, type of binder, binder concentration, working conditions (temperature, rpm and mixing time), drying process and medium of suspension (acidic/basic). All these parameters are interlinked with each other. During drug release application the more prominent mechanism that governs/controls the release rate of drugs is diffusion and swelling/erosion or both mechanisms. The mechanism and the characteristics of various drugs encapsulated with chitosan and its derivatives are presented in Table 1.

2.1. Effect of molecular mass of chitosan on drug release

The molecular mass of a polysaccharide has been regarded as one of the significant factors which could affect its functionality. Investigations conducted by different researchers on the MM of Chitosan reveal its significance in terms of anti–microbial, anti–fungal, DD (in terms of interactions with drugs) and drug release properties. Different methods have been used for the determination of MM of CS including osmometry, end group analysis, light scattering, sedimentation, gel permeation chromatography (GPC), Qin, HPLC and viscometry techniques etc [51,52]. The osmometry technique is one of the classical cost–effective methods subcategorized in to vapour pressure osmometry (VPO) and membrane osmometry (MO). Among these two subcategories the VPO method is more suitable for polymers having number average molecular mass (*Mn*) less than 20,000 g/mol. The MO method faces a problem due to insufficient control over the diffusion and molecules of LMM passes through membrane area and resulting high uncertainties. This method mainly depends on the colligative characteristics of the polymer solution and this solution is separated from the solvent through the membrane due to chemical potential [53,54]. The end group analysis is considered as a significant cost–effective method especially for determining the average molecular mass (AMM) [55,56]. This technique requires a highly pure polymer for analysis and process is based on some assumptions along with chemical or spectroscopic techniques. This method can only be effective for the polymers if their MM is less than 20,000 amu, and the polymer concentration is high. Light scattering technique is a simple and popular method for the determination of the AMM of the polymer [57–59]. The AMM measurements are based on the quantification of the elastic light scattering and the advantage of using this method is that it can measure a wide range of MM (normally 10^4 to 5×10^6 g/mol) as well as polydispersity. Sample preparation is one of the complexities of this method. Some of the researchers suggested the use of the size exclusion chromatography (SEC) due to high reproducibility of estimated MM of CS. This method can estimate AMM (Mn, MM and polydispersity index) just in a single step [60–62]. Similarly, the GPC has become one of the preferred methods for the estimation of MM as well as polydispersity of polymers [63–69]. The highlight of this method is that it can determine AMM in various ranges (Mn, MM, Mz and M_{z+1}). However, some complexities are involved with this method in terms of sample preparation, time and the high cost of equipment. Viscometry is also a cost–effective method for determination of MM of polymers [70–73]. This method is somehow less accurate, time taking and has limitations for LMM polymers. All these methods are worthy in the estimation of molecular mass but seem to have certain limitations which sometimes make them unattractive to be used for various polysaccharides. The chemical reactions such as oxidation, derivatization, and depletion modify the polysaccharides with a compromise of lowering in MM which sometimes cannot be estimated by any ordinary technique. In this regard, several NMR spectroscopic techniques along with computational procedures have been developed for the better characterization of polysaccharides [74]. Diffusion–ordered (Dosy) is one of the simple and fast NMR techniques which can be used for the estimation of intermolecular interactions, and AMM for various polymers, polymer blends and mixtures [75,76].

Since most of the CS applications depends on the protonation of amine groups into $–NH_3^+$ in acidic medium. This degree of protonation (pKa) is dependent on the MM of CS and it slightly decreases with a decrease in the molecular mass of CS. In this regard different authors have investigated and reported different pKa values for a given CS sample [77,78]. The changes in molecular mass of CS can alter its pKa values, which in turn might change its protonation ability, surface charge and stability, hence, affecting the drug delivery and release applications. The particle size and surface charge of CS are those characteristics which directly influence the drug release applications. It is considered a big challenge to develop the size, shape and surface charge-controlled CS particles as only the correct evaluation could find the significant relation of these physiochemical properties with the drug release applications.

The understanding of the interaction between polymer and proteins is essential for the development of drug release applications. This could give an idea of how strong or weak binding forces exist between them. The thermodynamic parameters such as ΔH and ΔS can provide the nature of interactions, whether they are hydrophobic or electrostatic. If $\Delta H > 0$ and $\Delta S > 0$, then hydrophobic interactions are dominant, if $\Delta H < 0$ and $\Delta S > 0$, then interactions are due to electrostatic attraction, and finally if both $\Delta H < 0$ and $\Delta S < 0$, then both hydrogen bonding and van der Waals forces are responsible for interactions. Likewise, protonation degree (pKa) the interaction between polymer

and proteins is also dependent on MM of CS. A recent study correlated the MM of CS and binding affinity of this polymer with two proteins (BSA and HSA). In this regard, CS with a constant DDA, and with different MMs (15, 100 and 200 kDa) was investigated. The results indicated that CS NPs interacted with BSA and formed a CS–BSA conjugate through hydrophobic interaction. For the case of CS–BSA, the sequence was found to be 200 > 100 > 15 kDa. This reveals that stronger interactions between polymer and a protein can be developed by increasing the MM as seen in case of CS of 200 kDa. In the case of HSA, the electrostatic contact was more dominant to form CS–HSA conjugate. The order of interaction for CS–HSA was found for the MM in the sequence of 100 > 200 > 15 kDa, which reveals that CS–HSA having MM 15 kDa possessed weak binding forces as compared to MM 100 kDa. The less availability of charged sites as compared with CS–HSA conjugate of MM 100 kDa could be the possible reason [79]. These stronger interactions will ultimately result in better release profiles.

Polyethylene Glycol (PEG) has also been reported as a potential carrier in the drug release applications [80]. PEG has been blended with CS for encapsulation/coating of drugs and resulted in more stable NPs. The interactions between PEG and CS are weak but have countable effects on the physiochemical properties of NPs (size, shape, encapsulation efficiency and drug release). These interactions are the results of intermolecular hydrogen bonding of hydrogen from CS and oxygen from PEG [37,81,82]. Some studies revealed that the addition of PEG to CS did not significantly improve the mechanical strength but their incorrect combinations (CS: PEG ratios) resulted in the reduction of mechanical strength [83]. Chitosan along with Katira gum has improved the anti–inflammation action and drug release profile of Glycyrrhizic acid (GA). The experiments conducted in PBS (pH = 7.4) at 37 ± 0.5 °C and 100 rpm showed a sustained release of GA (90.71% in 12 h) from GA–Chitosan–gum NPs. Addition of gum to chitosan resulted in a controlled release of GA as shown in Fig. 2.

The main reason for such a controlled release may be due to the selection of pre–optimized conditions including the concentration of CS (0.21% w/v), GA (50% w/w of CS and gum) and katira gum (0.005% w/v). These optimized conditions resulted in NPs with smaller size and smooth surface, which has a very high zeta potential (41.0 mV). These properties (size, shape and zeta potential) are in good agreement with their predicted properties. These modified CS NPs exhibited good release characteristics following two mechanisms (diffusion as well as erosion of polymer). The data fitting of drug release rate into various models; zero order, first order, Higuchi and Korsmeyer–Peppas resulted in R^2 values of 0.996, 0.927, 0.929 and 0.997 respectively. From the model fitting analysis, the release rate of GA from the CS–gum–GA NPs was found to follow zero order kinetics. The approach used in this system indicated the importance of the optimization of parameters for controlling the release mechanisms [84].

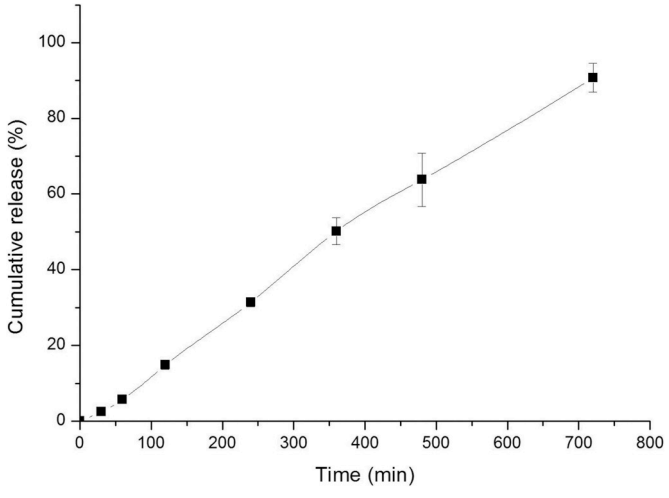

Fig. 2. Release profile of the Glycyrrhizic acid from GA–Chitosan–gum NPs (Reprinted with permission from Ref. [84], Copyright (2016) Elsevier B. V.).

The particle size increases with an increase in MM of Chitosan and the general sequence of particle size is: HMM > MMM > LMM [85,86]. Control over the MM of CS can control the size and shape of particles and hence, ultimately resulting in better drug release. This factor affects certain properties of CS because its reactivity changes as the MM changes from low to higher kDa. Usually a higher diffusion rate is expected from LMM CS as compared with HMM CS [87]. In the case of particle size, preparation technique is also a factor which affects the particle size when CS of different MM is used. For example, when the CS particles are spray dried, their sizes tend to increase with an increase in their MM. Moreover, the higher the MM of CS, more uniform and spherical shaped particles are formed. At HMM of CS, the viscosity is higher and narrow particle size distribution is achieved which leads to the formation of uniform particles. Similarly, low viscosity may lead to the formation of irregularly shaped particles due to the droplet shrinkage. The CS chemistry changes with the addition of a substance and leads to defer the relationship with MM and particle size [88]. Similarly, the swelling of particles/tablets is dependent on MM of CS. The swelling property increases as the MM decreases due to the fact that media is also responsible for this swelling. This statement has some limitations, for acidic medium valid up to 120 min and after this, HMM CS possess higher swelling up to 360 min and for phosphate buffer saline (PBS) having pH = 6.80 up to 360 min). The CS particles in acidic medium (0.1 M HCl) swell more than that in PBS at pH = 6.80. In this kind of matrix, swelling and erosion properties of the particles are more prominent and control the drug release mechanism (DRM) in combination with diffusion mechanism. A drug release study of Propranolol from the Chitosan–magnesium aluminum silicate (CS–MAS) tablets in two different mediums (acidic medium 0.1 M HCl and PBS having pH 6.8) was conducted and the release mechanism was determined by fitting the data into two models (zero–order (k_0) and Higuchi (k_H) models). The results revealed that release of propranolol from the matrix in acidic medium was independent of MM of CS and even HMM CS with and without MAS exhibited higher drug release after a certain time. The data for drug release was best fitted with zero order (k_0) model as compared with Higuchi (k_H) model. Therefore, the drug release mainly occurred due to swelling and erosion along with diffusion. In this case, the MM of CS is not directly responsible for drug release, but the swelling is the main cause for its release. Higher swelling resulted in a longer available path for diffusion of drug molecules and hence reduced the drug release through this path. On the other hand, the drug was released at a sustained rate of up to 8 h in PBS having pH = 6.80. In this case, the DD was dependent on the MM of CS and minimum drug release rate (K_0) was seen for HMM CS. In this case, slow swelling and high crosslinking of CS with Phosphate helped in sustaining the polymer-drug matrix and hence resulted in low drug release [89]. Different findings and different opinions create conflicts among the researchers. Some of them reported that the drug release from this kind of tablets is faster for HMM CS [90]. The reason for these conflicts may be the purity of CS, MM, DDA, process parameters/conditions, working environment and equipment conditions. These conflicts make the researchers and scientists work hard and explore the deep understanding of materials and their interaction with other substances, which ultimately results in better formulations. Chitosan NPs have been used for the encapsulation of different drugs for various treatments [91,92].

Ammonium glycyrrhizinate (AG) is an anti–tumor and anti–inflammatory agent which has poor absorption when administered through the oral route. Since CS has good encapsulation efficiency and proven to enhance the absorption of several drugs, it was used for the modification of AG. The MM, concentration of CS and PEG significantly affected the physiochemical properties and encapsulation efficiency of

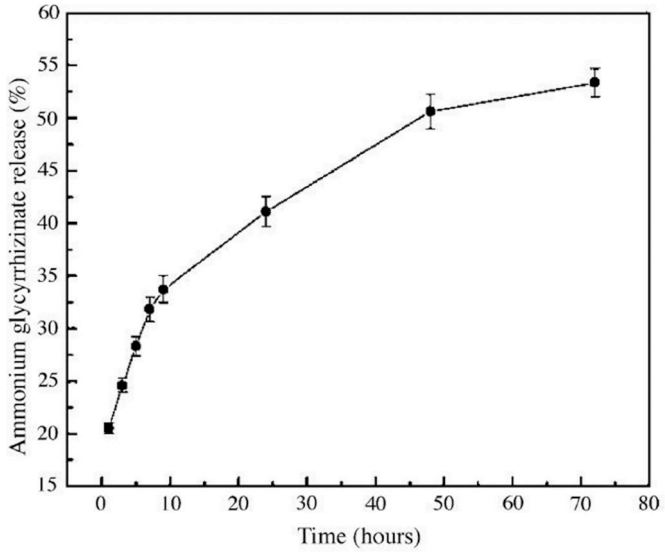

Fig. 3. Release of Ammonium glycyrrhizinate (AG) from CS–PEG–AG NPs (LMM Chitosan) (Reprinted with permission from Ref. [81], Copyright (2005) Elsevier B. V.).

CS–PEG–AG NPs. Chitosan with HMM interacts efficiently with AG and resulted in high encapsulation efficiency as compared with LMM CS, while the increase in the concentration of CS or AG results in low encapsulation efficiency. Usually, the positively charged CS interacts with negatively charged AG through ionic gelation, whereas, the addition of PEG to CS before adding the AG creates a sense of competition among the PEG and AG to interact with the Chitosan. The release profile of AG from the LMM (24 kDa) Chitosan–AG NPs exhibited a burst release initially, and afterward showed a slow release (Fig. 3).

Two types of release mechanisms (diffusion and polymer degradation) were involved. The reason behind the burst release might be due to the desorption of drug molecules from the NPs surface. Another hypothesis may be that the AG molecules being smaller than NPs may easily diffuse through the pores of nanoparticles and hence resulting in fast drug release [81]. On the other hand, the polymer degradation resulted in the slow release of AG from the modified NPs. In this case, the role of PEG with respect to physiochemical properties is clear but its role in drug release was not investigated. The better understanding of AG release from CS and PEG–CS NPs could be achieved by investigating the role of PEG and MM of CS.

Based on the above discussions, it is clear that various properties are dependent on the MM of CS, which can ultimately affect the drug release applications. Among these properties, reactivity and interaction of CS with drugs and other materials, swelling and drug diffusion play important role in controlling the release of drugs. Before analyzing the importance of MM of CS in drug release, it would be necessary to highlight that CS is usually obtained from different sources and different methods are applied for the determination of its MM. Some of these methods have limitations and high uncertainties which may result in inaccurate MM. Under such circumstances, CS properties such as pKa value could be different ultimately the optimization based on MM could be less efficient/accurate. The determination of MM of CS should be reproducible. Further, reactivity and interaction of CS with the drug is responsible for controlling the release characteristics. These interactions increase with an increase in MM of CS. Different cases attributed different types of CS-drug interactions such as electrostatic and hydrophobic. In some cases, electrostatic interaction resulted in more controlled and sustained release of drugs as compared with hydrophobic interaction for the same MM, however the deep understanding of these interactions is missing. The additional information on the CS-drug interaction could provide a better control over release profiles. Among the studied materials, BSA and PEG had the hydrophobic, while HSA, Katira gum and AG had electrostatic interaction with CS. There may be cases, where the drug molecules are so small that they can easily penetrate through the polymeric matrix even if the CS possess HMM.

2.2. Effect of degree of deacetylation on drug release

The Degree of Deacetylation of CS can be determined by various techniques including elemental analysis, Titration, Hydrolytic, HPLC–UV and NMR techniques [93–98] etc. Several advantages and disadvantages are involved with the selection of these methods which ultimately provide incomparable DDA results. The estimation of DDA by using elemental analysis is not considered as a precise method because this method is based on the percentage of nitrogen content in completely deacetylated CS. Using this method, different CS samples with variable DDA values presented small variations in nitrogen contents and hence, resulted in inaccurate results/predictions. Titration methods are subcategorized into acid–base, potentiometric, colloid and conductometric titrations. In acid–base titration the DDA values are estimated from the titration curve with the help of inflection points. In this method, the viscosity of the sample solution and the formation of precipitates are responsible for the accuracy of the results. In potentiometric titration the DDA values are estimated by the measuring the number of amino groups at two inflection points. This method needs special care in terms of purity and weight of the CS sample. This method is not suitable for measuring DDA of the low–grade CS samples [99]. In the case of colloid titration, the DDA measurements are carried out using stoichiometric calculations based on the ions. This method is not suitable for the samples having low DDA as the end point is difficult to find/locate and in some cases neither colour change nor precipitation occurs [100]. On the other hand, the conductometric titration is applicable to CS samples having low DDA as compared with high DDA samples due to the low solubility of CS in HCl [93]. In this method the solubility of CS sample is essential for the estimation of DDA [101]. The hydrolytic method is the cheap method which requires simple chemicals for the estimation of DDA, but its procedure is somehow complex as multiple steps (hydrolysis, acidification and distillation) are involved [102]. The HPLC–UV is a spectroscopic technique used for the estimation of DDA of CS samples. This is an affordable method which can also determine the MM of CS. This method is applicable to the acidic acid soluble samples and special care is required for the preparation of CS samples and calibration [103,104]. Gas chromatography is also a useful method for the estimation of DDA. The benefit of this method is that it works for samples of entire DDA range. It could be used as a routine analysis as it requires a small sample with less time for testing. Adaptation of this method sometimes may results in inaccurate results due to contaminations caused by the carbohydrates [105]. Nuclear Magnetic Resonance (NMR) spectroscopy is nowadays widely used for the estimation of the DDA of CS as it is reliable than other techniques and its results are reproducible [106]. The analysis can be carried out with a small sample in a short time. This technique is subcategorized into liquid state and solid state analysis [98,107–111]. Liquid state analysis takes short time and a small sample is enough whereas, solid state analysis is more expensive and time consuming. The later analysis sometimes provides nonreproducible results because of the contaminations caused by carbohydrates whereas, the advantage of this method is that it can estimate DDA values of insoluble samples which is lacking in liquid state analysis. This technique is very much effective for characterization of modified CS and interpretation is easy as compared with techniques discussed before. Moreover it is a non destructive technique and the samples can be recovered back after analysis.

The degree of deacetylation (DDA) is one of the important factors which govern the changes in properties (solubility, crosslinking, particle size, shape, degradation and release profile of the drug) of CS and make it suitable to be considered for various targeted applications. The

degradation rate and radiation stability of the CS depend on the crystallinity which in turn depends on the DDA. In this case, the increase of DDA ratio results in low crystallinity of CS and it possess a loose structure which in turn results in high degradation rate. A study conducted on the radiation degradation of CS proved its importance [112], indicating the degradation rate decreases as the crystallinity of CS increases. In another study of CS degradation behaviour with respect to DDA and MM, the results showed that higher DDA values slow down the degradation of CS as compared with low DDA values for samples with similar MM [113]. The solubility of CS in acetic acid, crosslinking of CS with a suitable crosslinking agent and hydrophobicity of microspheres were found to increase with an increase in the DDA. The addition of free amino groups, due to increasing DDA, changes the properties of CS and helps to attain more solubility and better crosslinking without a change in MM [114]. This factor also affects the size and morphology of particles, ultimately play a role in the release profile of the drug from the matrix. A study conducted to investigate the effect of DDA (90% and 75%) of CS on particle size, encapsulation efficiency and release of the anti–metabolite drug Fluorouracil (5–FU) with CS NPs proved that the particle size decreases as the DDA values of CS increases, while encapsulation efficiency increases with the increase of DDA values of CS. The release profile of the 5–FU in PBS (pH = 7.40 and at T = ± 37 °C) showed a burst release (≥55%) in 10 h, and a sustained drug release (≥75%) in 100 h from the 5–FU–CS NPs prepared by 90% DDA CS. On the other hand, the 5–FU–CS NPs prepared by 75% DDA exhibited initially a burst release (≥75%) in 10 h and a sustained release (≥85%) in 100 h. The electrostatic interactions are based on the charge density and in this case the charge density of the NPs prepared with the higher DDA CS (90%) was found to be higher. The strong interaction between the CS and polyanionic TPP lead to a slow and sustained release of 5–FU [113]. The release profiles of 5–FU from 5–FU–CS NPs (CS having DDA = 90% and 75%) are shown in Fig. 4.

As discussed earlier, the size, shape, surface charge, zeta potential and type of interaction between the CS and drug are the significant factors which have the ability to change the release profile, which in turn are dependent on DDA of CS. The DDA value of CS is usually determined by the various methods which have certain limitations and sometimes results in incomparable DDA values. Therefore, it would be necessary to consider that the DDA value of same CS sample may have different numbers depending on the method of determination and hence resulting in a different release profile for the same CS sample. The higher DDA values of CS result in more spherical and compact NPs which degrade slowly and ultimately results in slow drug release. It is important to consider the effects of DDA while formulating the CS-drug matrix for controlled release applications especially if a higher degree of drug adsorption is required. The high drug adsorption on to the NPs can be achieved by using the higher DDA CS.

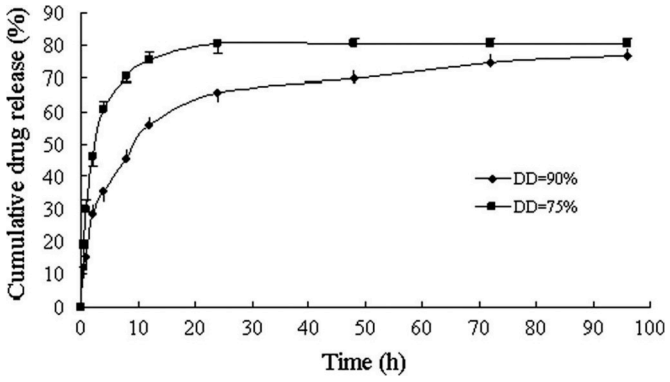

Fig. 4. Release profiles of 5–FU from 5–FU–CS NPs (CS having DDA = 90% and 75%) (Reprinted with permission from Ref. [113], Copyright (2010) Taylor & Francis Group LLC).

2.3. Effect of initial pH of chitosan on drug release

The CS NPs/MPs face serious issues of mechanical/colloidal instability which results in burst release of drugs and minimizes their advantages to be used as a drug carrier in controlled release applications. The burst release of drugs can be partly controlled by controlling the mechanical/colloidal stability of CS NPs/MPs. The optimized encapsulation of anti–cancer drug PTX with Glycol Chitosan–Hydrotropic Oligomer NPs (GCS–HO NPs) improved the therapeutic efficiency of PTX by enhancing the drug loading capacity. The initial pH of the CS solution is a significant factor which can affect the stability of CS NPs/MPs and hence affecting the drug release rate. Therefore, it is necessary to optimize the initial pH of CS solution to prepare the mechanically stable NPs/MPs. A sustained release of PTX was seen from the PTX–GC–HO NPs based on PTX initially added concentrations (10, 20 and 30 wt %). The PTX release from the formulations having (20 and 30) wt. % PTX showed much slower than the formulation having 10 wt % PTX (Fig. 5). The slow release was due to the better stability of PTX–GC–HO NPs which is surrounded by the HOs [114].

Some researchers have indicated the critical pH limits for the formation of NPs. Initial pH of CS solution can affect the size, shape, zeta potential, stability, yield and other properties of CS NPs. In ionic gelation method, the CS NPs are prepared by the interaction of CS with TPP which is mainly based on initial pH of the solution. In this regard, the different effects of initial pH of the CS solution have been indicated over the particle size, shape, zeta potential/stability and drug release rate [85,115–118]. A study conducted on the preparation of CS–TPP NPs indicated that the size of the particles and zeta potential decrease as the initial pH of CS solution increases. Smaller particles are expected to form at pH = 4.0–5.0, and afterward larger particles, whose size will increase sharply partially depending on the concentration and MM of CS. At this pH range the $–NH_3^+$ ions are less neutralized resulting in the formation of small particles during crosslinking. The better understanding about the initial pH of CS solution can be obtained by categorizing it into three zones. The first zone (pH = 3.30–4.50) where CS is strongly protonated into $–NH_3^+$ groups and possesses strong repulsions which lead to the formation of larger particles. The second zone (pH = 4.5–5.0) is considered as an optimum zone for the formation of small particles due to appropriate crosslinking. Finally, the third zone is considered as the aggregation zone where initial pH of CS solution is almost equal to pKa value (6.5) of CS. Different researchers have different opinions regarding this zone depending on their range of investigations and findings. Some of them suggested that agglomeration occurs after pH = 5.0, while some of them considered that it occurs after pH = 5.50 [119]. Fan et al. (2012) investigated the significance and critical limits of initial pH of CS solutions for the preparation of CS–TPP NPs and explained that NPs are difficult to produce below the pH = 4.50, while the pH is > 5.20, the MPs are formed [115]. Their

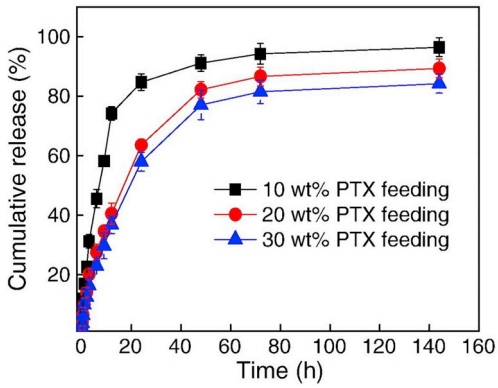

Fig. 5. Release of PTX from three different formulations of GCS–HO NPs (Reprinted with permission from Ref. [114], Copyright 2013 Elsevier B. V.).

findings revealed that the initial pH of the CS solution affects its protonation ability as it decreases from 100% to 0% while changing the initial pH of CS from pH = 4.7 to pH = 8.0. This provided the evidence of occurrences of the critical limitations in the formation of NPs and their stability with respect to initial pH of CS solutions.

On the basis of stability study conducted with PBS having pH = 7.40, the CS NPs formed by CS solution with an initial pH = 4.0–5.0 are considered more stable as compared with CS NPs formed at an initial pH = 5.50 (precipitated in 1 h). On the other hand, slight changes in stability behaviour have been noticed, when the CS NPs prepared with an initial pH of 4, 5 and 5.5, which were diluted in same pH medium. This indicated that the stability of CS NPs is dependent on both pH of medium and initial pH of CS solutions [120]. The change in initial pH of CS or pH of a crosslinker can also alter the release rate of drugs. Bhumkar et al. (2006) highlighted the effects of initial pH of Tripolyphosphate (TPP) on crosslinking property of CS with TPP. The change in pH of TPP arises a competition among the Triphosphorus Pentoxide (P_3O^{5-}) and OH^- ions to interact with CS, ultimately affecting the extent of crosslinking. The higher pH value of TPP (pH = 9) minimize the crosslinking of CS with P_3O^{5-} due to deprotonation of CS and hence, as a result OH^- ions also interacts with CS. On the other hand, at low pH of TPP (pH = 3) only P_3O^{5-} ions crosslink with CS through ionic interaction [121].

The solubility of CS depends on its protonation state which in turn depends on the initial pH of CS. An optimized initial pH of the CS solution may form mechanically stable NPs or MPs which could ultimately dictate the release profile of drugs. It should be considered that pKa values of CS vary from 6.2 to 6.5 based on techniques used. This may shift the optimized initial pH of CS to some other value and results in high, moderate or less stable NPs/MPs. The selection of initial pH of CS in the range of 4.50–5.30 could form a stable and better crosslinked CS NPs/MPs for the encapsulation of anti-cancer, anti-diabetic or anti-infectious drugs. The crosslinking is highly dependent on the initial pH of CS and highly crosslinked NPs/MPs can improve the release profile of several drugs. Therefore, better understanding/knowledge of pKa values of CS and initial pH of solution is essential while designing or fabricating the drug formulations with CS or CS related conjugates.

2.4. Effect of pH of suspension medium on drug release

The pH of suspension medium is one of the important factors which governs the changes in the structure of CS leading to protonation or deprotonation which ultimately resulting in the change in size, shape, zeta potential/stability and release profile of the drugs. In low pH medium, the structure of CS expanded due to the significant repulsions caused by highly protonated amino groups. Whereas in higher pH medium, the structure of CS gets shrink due to deprotonation and low repulsive forces. This pH trigger property of medium affects the drug release profile and high drug molecules are found to be released in a low pH medium than in a high pH medium.

Since both the physiochemical properties and drug release characteristics are dependent on the methods of preparation of NPs/MPs, pH medium, co–polymer/crosslinker and technique used for determination of release rate respectively. Based on these factors, it is always possible that the same drug may end up with different release profiles. Cyclosporin A (CyA) was encapsulated with a HMM CS (MM 120 kDa and DDA of 75–85%) and a co–polymer mPEG (MM 5000) by using emulsification/solvent evaporation method and the obtained CS–PEG–CyA NPs were found to have much smaller size (89.15 nm) as compared with the CS–CyA NPs (293 ± 9 nm) prepared by ionic gelation method. In this case, the addition of mPEG did not increase the size, but zeta potential was dropped to −8.5 mV which was high in case of CS–CyA NPs (37.5 ± 0.9) mV. For a medium with 0.10 M PBS (pH = 7.4) the dialysis technique was used while stirring at room temperature. As shown in Fig. 6, the release of CyA from the CS–mPEG–CyA NPs was around 32.6% in 48 h, whereas the release

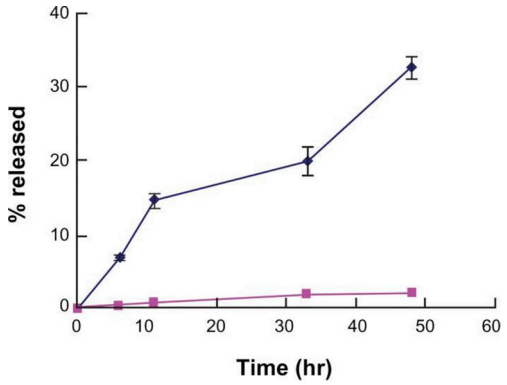

Fig. 6. The release of CyA: (♦), from CS–mPEG–CyA and (■), from CyA saturated solution (Reprinted from Ref. [122], open permission for non-commercial use Copyright (20013) Dove medical press).

from CyA saturated solution was only 4% in 52 h. This limited release was attributed to the poor solubility of CyA which was improved by the addition of CS–mPEG NPs. The kinetics of the drug release highlighted that the drug release was based on bulk erosion mechanism [122].

The release profile of CyA indicated that the presence of a co–polymer like mPEG can enhance its solubility in 0.1M PBS medium (pH = 7.4 and using the dialysis technique) ultimately resulting in more drug release. Similarly, the release profile of a sparingly soluble drug, salazosulfapyridine (SASP) was improved by their modification with 2,6–dimethyl–cyclodextrin–Chitosan (DMCD–CS) NPs under the optimized parameters (medium pH = 6.80). The release of SASP in pure form was low (10%) in 4 h, and (approximately 15%) in 24 h, while the modified NPs exhibited a fast release (approximately 42% in 4 h) and a sustained release (approximately 75%) in 24 h (Fig. 7) [123]. The release profiles of pure SASP and modified DMCD–CS NPs showed that these kinds of carrier materials may provide the biphasic release profiles for the less soluble drugs.

In another study, the Podophyllotoxin (PPT) encapsulated with CS NPs was exposed to different pH mediums at 37 °C. Their results indicated the dependency of these particles on the medium pH and it was found that PPT release increased with a decrease in medium pH. The release of PPT was due to drug desorption from the surface of NPs (a fast release of around 15–20%), some extent of drug diffusion and

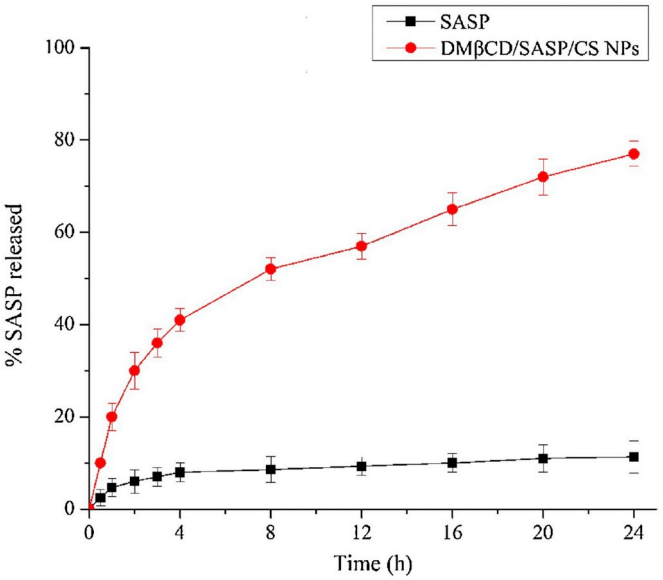

Fig. 7. Release of SASP from: ■, pure form and (●), SASP–PDMCD–CS NPs (Reprinted with permission from Ref. [123], Copyright (2017) Elsevier Ltd.).

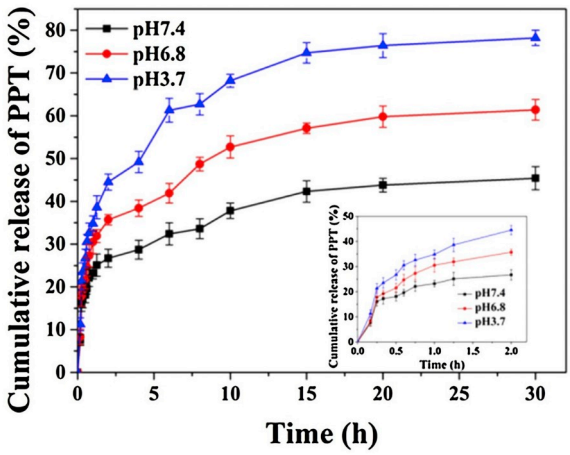

Fig. 8. Release profile of Podophyllotoxin from CS NPs in different pH mediums (Reprinted with permission from Ref. [124], Copyright (2017) Elsevier B. V.).

breaking of hydrogen bond due to protonation of amino groups. Around 78% of PPT was released in 30 h at pH = 3.70 which was much higher as compared to that at pH = 6.80 (60%) and pH = 7.4 (45%), and the trend of PPT release is shown in Fig. 8. The reason could be due to the shifting of the intact structure of CS into a loose structure, at a pH less than the pka value of CS [124].

Chitosan has some limitations such as high hydrophilicity, low solubility in a basic pH medium (pH = 7.40), low ductility, a high degree of swelling and thermally less stable. Surface modification of CS with different derivatives helps to overcome these limitations. Quaternization, acylation, thiolation and carboxymethylation are the common chemical techniques used for the modification of CS for targeted pharmaceutical application [125]. Chitosan with its carboxymethyl derivatives is well known due to its non–toxicity, biodegradability and enhanced drug bioavailability. This modified form of CS has better control over the release of drugs. Carboxymethyl Chitosan (CMCS) has been used with other co–polymers including alginate, PLGA, PLA, PEG etc for modifications of various drugs and has in vitro potential for sustained release applications [126]. Modification of the anti–cancer agent like Curcumin with CMCS NPs showed a specific toxic efficacy for the cancer cells but remained non–toxic to the living cells and its drug release profile revealed that the Curcumin was released from modified chitosan NPs with a sustained rate. The release, in this case, depends on the medium pH, which might be due to the swelling of the polymeric complex. The amine group present in N–O–CMCS NPs gets protonated in the medium (pH = 4.5), which results in a repulsive force between the adjacent positive charges. This repulsive force increases the swelling of polymer which in turn increases the drug diffusion rate. In the basic medium, the force of attraction is more prominent and limited swelling occurs which leads to a low diffusion rate [127]. The release profile of insulin from the same material CMCS also showed that insulin encapsulated in this carrier material is dependent on the pH of the medium. In acidic pH medium (pH1.2) the insulin release is lower (64%) as compared with pH medium 6.8 (90%) after 2 h. The sustained release in acidic and basic mediums was obtained as the CMCS and binder $CaCl_2$ ratios increase from 10:3 to 10:7 in the drug formulation. The later ratio provided the most optimized combination of CMCS-$CaCl_2$ to control the release of insulin. The high concentration of the binder increased the strength of NPs as a result of suitable crosslinking density available for insulin towards NPs formation which is absent in other combinations (10:3 and 10:5) [128]. The characteristics of the CMCS are influenced based on their sites formation such as O-CMCS, N-CMCS and N,O-CMCS. Different drug release profile could result from these carboxymethyl Chitosan sites. Study of these CMCS NPs indicated that O-CMCS is the most desirable carrier material among three types and has better control of the release of Docetaxel (DCT). In PBS medium (pH = 7.4), these CMCS NPs exhibited an initial burst release of 40–50% in 10 h afterward following a sustained release (62.6–84.9%) for 3 days [129].

Curcumin as the drug for the purpose of anticancer treatment was modified with Chitosan mesoporous silica nanoparticles (CS–MCM–41 NPs). The modified drug NPs were prepared by using MMM CS with DDA value of 75–85% through sol–gel method which influenced the release profile of Curcumin from NPs. This drug loaded and modified NPs were found to be pH responsive and showed that the addition of Chitosan as a shell material to the mesoporous silica–based material MCM–41 NPs resulted in a controlled and slow release of Curcumin in the acidic medium as compared with MCM–41 based NPs. In the acidic pH medium (pH = 5.50), the CS modified MCM–41 NPs exhibited a release of Curcumin (33.94 ± 2.42% for 32 h) and (42.72 ± 2.29% for 96 h) respectively, whereas in PBS medium (pH = 7.40), Curcumin release of 16.85 ± 1.17% from the CS modified NPs and 33.94 ± 2.42% from the unmodified MCM–41 NPs was found for 32 h. For a long run of 72 h, the CS modified NPs exhibited a release of 19.54 ± 1.36% Curcumin while, the unmodified MCM–41NPs resulted in the release of 39.91 ± 1.26%. The combination of MCM–41 with CS resulted in a controlled and sustained release of Curcumin as compared with CS or MCM–41 alone [130]. Similarly, the Curcumin release from the Folate–Chitosan modified nanoparticles (F–CS NPs) exhibited pH dependency as the release of Curcumin increased with the decrease of medium pH from pH = 7.40 to pH = 5.0. The release profile showed a burst release of Curcumin in 3 h in both the mediums of pH = 7.40 and

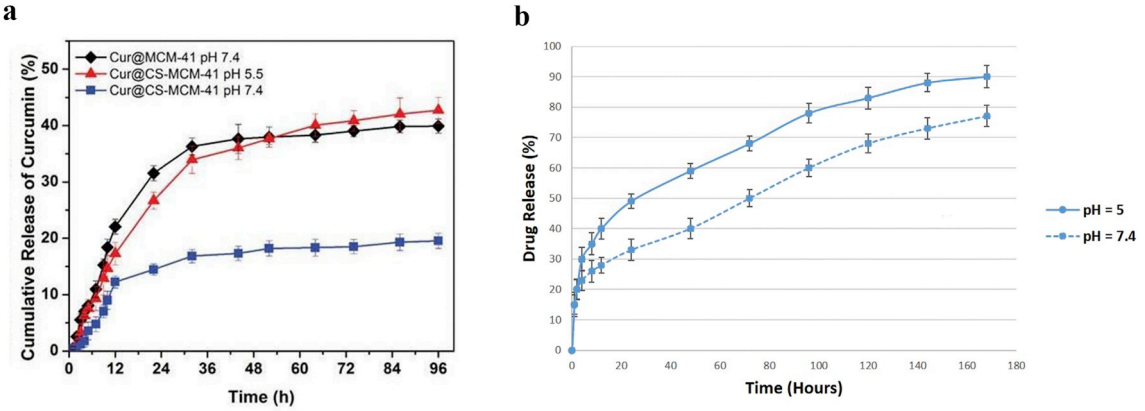

Fig. 9. Release profiles of Curcumin from: (a), (♦), MCM–41 at pH = 7.40; ▲, CS–MCM–41 pH = 5.50 and ■, CS–MCM–41 in pH = 7.40 (Reprinted with permission from Ref. [130], Copyright (2018) Taylor & Francis Group) and (b), F–CS NPs in two different pH mediums (pH = 5.0 and 7.40) (Reprinted with permission from Ref. [131], Copyright (2017) Elsevier Ltd.).

5.0 followed by a controlled release over one week (75% and 90% for pH = 7.40 and 5.0 respectively). The main reason behind the higher Curcumin release could be due to the selection of in vitro drug release method, where the parameters were based on the direct dispersion of modified NPs in both pH mediums (pH = 7.40 and 5.0). In addition, both the shaking and centrifugation at higher speed could be responsible for the higher release rate [131]. The release profiles of Curcumin from CS–MCM–41 NPs and F–CS NPs are shown in Fig. 9 (a and b).

Few literature indicated that stability was affected by the MM of CS. The NPs prepared by HMM CS showed a better colloidal stability as compared with that prepared by MMM and LMM CS. While dissolution properties of Curcumin under sink conditions are not significantly affected by the change in MM of CS [132]. The Doxorubicin (DOX) an anti–cancer drug modified with Chitosan and its derivative O–Carboxymethyl Chitosan nanocomposite (CS/CMCS NPs) improved the bioavailability of DOX up to 42%, while the release was initially burst followed by a slow release [78]. Burst release of DOX from the CS NPs was reduced further by modifying the DOX with CS–Clay NPs. The release of DOX was found in the following order: CS > CS–Clay (10:1) > CS–Clay (5:1) > Clay. In the case of Clay or CS–Clay NPs suspended in low pH medium (pH = 1.20), the positively charged DOX molecules bind strongly with the negatively charged Clay and resulted in slow release. On the other hand, suspension of these NPs in higher pH medium (pH = 5.30), CS has limited solubility, whereas these NPs show immiscibility at a medium pH = 7.40. As a result, the release of DOX from CS NPs decreased from 90% to 20% at low pH (pH = 1.2) and 15% at moderate to higher pH (pH = 5.3 and 7.4) respectively after 10 h. The rate of DOX release increased with an increase in CS contents in NPs. Therefore, optimization of the CS contents in CS–Clay NPs really helps in tuning the release rate of DOX [133]. Another study revealed the pH dependency of DOX release from the modified CMCS NPs. The in vitro release profile of DOX showed a release of 24.2% at pH = 7.4, and 54.8% in a medium pH of 6.0 and 78.8% in a pH medium pH of 5.0 for a period of 120 h. Further modification of the Carboxymethyl Chitosan (CMCS) NPs with 3–carboxyphenylboronic acid (CPBA) slightly reduced the release of DOX from the modified CBPA–CS NPs but this decrease was not significant. In this case, the DOX showed a release of 22.0%, 47.8% and 69.20% in a medium pH of 7.40, 6.0 and 5.0 respectively up to 120 h. An increased drug release was noticed when pH of the medium was changed

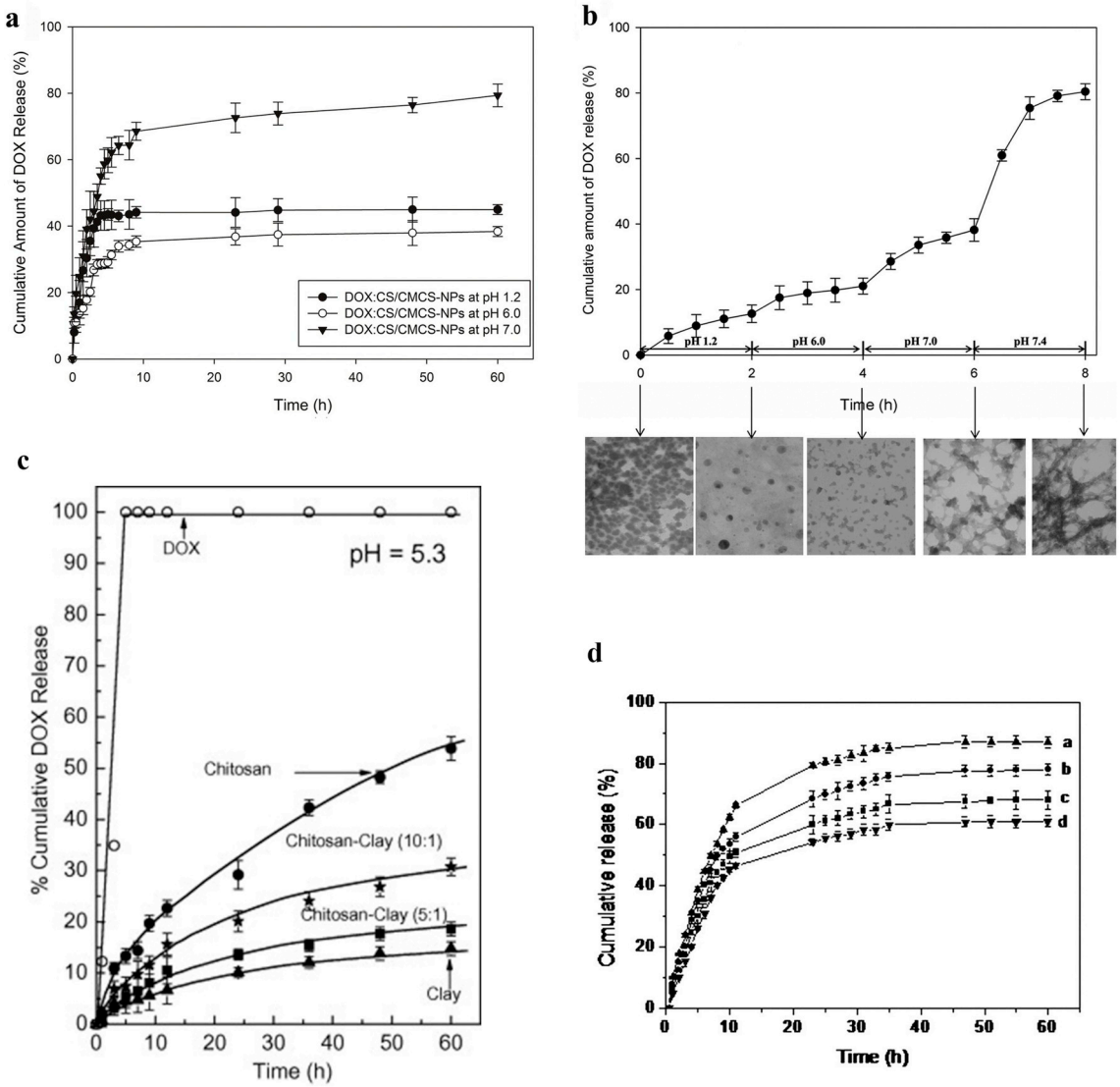

Fig. 10. Release profile of DOX from: (a) and (b), CS/CMCS NPs in different pH: ▼, pH = 7.0; (●), pH = 1.2 and (○), pH = 6.0 (Reprinted with permission from Ref. [78], Copyright (2013) Elsevier B. V.); (c): (▲), Clay; (■), CS–Clay (5:1); (*), CS–Clay (10:1); (●), pure CS and (■), pure DOX (Reprinted with permission from Ref. [133], Copyright (2010) Elsevier Ltd) (d); CS–PAMPS NPs at different pH mediums: (▲), pH = 8.0; (●), pH = 7.40; (■), pH = 6.0 and (▼), pH = 5.0 (Reprinted with permission from Ref. [136], Copyright (2016) Elsevier B. V.).

from pH = 7.40 to pH = 5.0 mainly due to the electrostatic interactions between DOX and modified NPs. These interactions became weaker with a decrease in pH and hence triggered the release of DOX. Further, the solubility of DOX in acidic pH as compared with basic pH medium is also responsible for this fast release [134]. The CMCS is one of the good derivatives of Chitosan with better water solubility, while hyaluronic acid has a good binding affinity for the cell specific surface markers along with other useful characteristics. Similarly, graphene oxide (GO) has obtained a considerable repute to be used as a carrier material in drug delivery systems due to its structure. Together they could provide better therapeutic effects as a carrier material for the controlled release of DOX. The release of (DOX) from the graphene oxide-carboxymethyl-hyaluronic acid (GO–CMC–FI–HA modified NPs showed a fast release (around 35%) in the first 12 h and afterward sustained release of 46% in 96 h, while in basic medium (pH = 7.4) it exhibited very slow release (12% only) in 96 h. The hydrophobic nature of DOX keep it deprotonated and results in π-π interaction with graphene oxide and hence results in limited DOX release. On the other hand, in acidic pH its ineffective reactive nature enhances the DOX release to meet the therapeutic requirements [135].

The conjugation of CS with PAMPS for the encapsulation of DOX also showed the pH dependent drug release. The initial burst release of DOX might be due to the physical adsorption of highly concentrated DOX on the outer surface of NPs. Unlike other conjugates of CS, the DOX release from these modified NPs was found to increase as the pH of the medium was increased from pH = 5.0 to pH = 8.0. The NPs were stable in acidic medium and showed limited release due to the protonation of CS and PAMPS groups, while the sulfonic acid groups of PAMPS deprotonated in the alkaline pH medium leading to the swelling of the NPs which ultimately increased the release of DOX. Similarly, the presence of CS disintegrated the NPs in the alkaline medium due to the deprotonation of ammonium groups which decreased the ionic interactions between CS and PAMPS, and hence favoured the release of DOX [136]. The release profile of DOX from various combinations can be seen in Fig. 10.

Modification of two anti–cancer drugs (PTX and DEX) with NPs of Glyceryl Monooleate (GMO) and CS resulted in the sustained release as well as the reduction of their side effects. The release profiles of both drugs revealed an initial burst release followed by the slow release (Fig. 11). The addition of Tween–80 (T–80) increased the release rate of PTX from modified CS–GMO NPs by increasing the water penetration. The reason for the initial burst release might be, either due to the drug molecules association with NPs or the swelling nature of CS in an aqueous medium. Therefore, the release rate of PTX and DEX can be tuned by controlling the water penetration [137].

Insulin an anti–diabetic drug has a serious issue of degradation on exposure to the acidic pH similar to GIT medium. Therefore, insulin exhibits a release profile based on pH of the medium. To improve the release profile of insulin several materials have been used as carrier material. Dextran (DS) and CS together have been used for the modification of several drugs [138,139]. When this combination was used

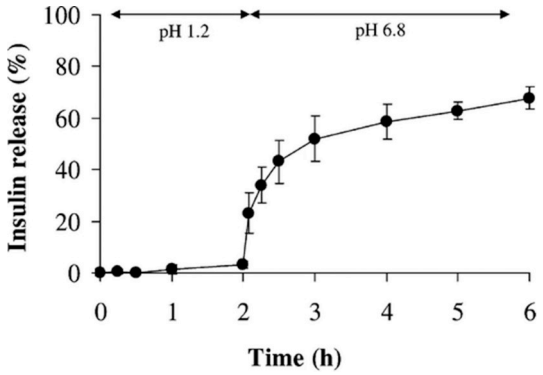

Fig. 12. Release profile of DS–CS NPs in two different pH mediums (Reprinted with permission from Ref. [140], Copyright (2007) American Chemical Society).

for the modification of insulin, its bioavailability was increased as compared with unmodified insulin. Further the loading of insulin with DS–CS resulted in stabilized NPs/MPs which in turn reduced the release of insulin by protecting it in acidic medium and most of the insulin was released in the basic pH medium (Fig. 12) [140].

The pH of medium affects certain properties of NPs/MPs (size, zeta, swelling index and interactions) which ultimately, affects the release profiles. Several drugs have the specific features of degrading in acidic medium and insoluble in basic medium. Therefore, it is essential to understand the shortcomings faced by the targeted drug so that a proper carrier material could be used for their fabrication. Likewise the drug features, the CS also swells in an acidic medium (due to protonation) and immiscible in basic medium (due to deprotonation) which creates the complexities in drug formulation. The control over the swelling and solubility of CS is essential to control the drug release from CS NPs/MPs. Fabrication of CS NPs with its derivatives (Carboxymethyl Chitosan, CMCS) and other conjugates (Clay and mesoporous silica etc has reduced the swelling in acidic medium ultimately slowing down the release. While in basic medium, these carrier materials provided a sustained but limited release of various anti-cancer drugs. The conjugation of folate with CS NPs would be more suitable for the encapsulation of poorly soluble drugs as it can provide high drug release rate in acidic as well as basic mediums. The modification of CS NPs with Poly(2–acry1amido–2–methylpropanesulfonic acid (PAMPS) have the ability to alter the release of drug as it can minimize the burst release in acidic medium and provide a sustained and prolonged release in basic medium. A number of materials have been used for fabrication of CS NPs as drug careers and they accelerated the release of drug in basic mediums as compared with un modified drugs, but many of these modified CS NPs were not investigated for their activity in acidic mediums. More investigations are required which may alter the release profiles of acidic degradable and sparingly soluble drugs.

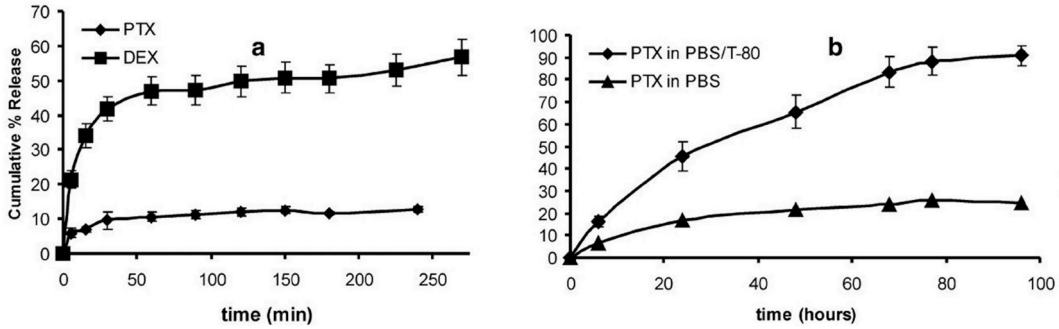

Fig. 11. Release profile of (a): (♦), PTX and (■), DEX and (b): (♦), PTX in PBS/T-80 and (▲), PTX in PBS (Reprinted with permission from Ref. [137], Copyright (2008) Springer Nature).

2.5. Effect of crosslinker on drug release

The release profiles of the various drugs not only depend on the DDA, but sometimes more optimized process conditions such as crosslinking of CS with a suitable crosslinker may results in better drug release profile even with a low DDA. This may slow down the drug release up to several hours avoiding the burst release of the drug. Till now many crosslinkers have been used for the formation of NPs such as dextran sulfate, glyoxal, genipin, Tripolyphosphate (TPP), formaldehyde and glutaraldehyde etc. Among these crosslinkers, the formaldehyde, glutaraldehyde and glyoxal are considered unsafe due to their toxicity and health issues (side effects). Therefore, a nontoxic crosslinker such as TPP is preferable for drug delivery applications. Using the TPP as a crosslinker provides the homogeneous crosslinking, hydrophilicity, biocompatibility and control on gel formation and hydration properties ultimately affecting the drug release both in acidic and basic mediums.

Several studies were performed using TPP as a crosslinker with CS which has improved the mechanical strength of NPS/MPS as well as release profiles of various drugs [81,85,86,92,113,115–121,141–146]. Oliviera et al. (2017) [147] also proved the importance of crosslinking and concluded that the TPP crosslinked CS MPs possess more sustained release characteristics whereas, the absence of crosslinking results in the fast release of the drug. A study using Isoniazid (INH) proved that the crosslinking had significant effects and it reduced the burst release. The non crosslinked CS exhibited a faster release (82% in 90 min for formulation CS: INH (1:2) which was even higher than that of INH solution alone (a burst release of 45%) without CS. This could be due to the change in crystallinity of INH which resulted in the diffusion of the drug and hence resulted in high INH release. In this case interaction of chitosan with INH without crosslinking might also be a factor for the acceleration of drug release. In this case, the change in (CS: INH) mass ratios did not affect the release of INH significantly. Further addition of the crosslinking agent to the same mass ratio (CS–TPP: INH), the release of INH was reduced ($\geq$50% INH release occurred within 6 h). Both the available amino groups and crosslinking prevented the drug molecules and hence, resulted in reduced drug release.

Chitosan NPs have intimate contact with corneal and conjunctive surfaces, resulting in improved ocular delivery to external tissues without any side effects. For the anionic drug, the CS (being a cationic polymer) is the best choice. The modification of the lipophilic drug Cyclosporin A (CyA) with CS (sea cure 123) by using TPP as a crosslinker resulted in a prolonged drug release of around 70% in 24 h under sink conditions. In this case, the initial release rate of CyA was higher mainly due to the dissolution of CyA nano–crystals associated with CS NPs, while a sustained release was seen afterward. The observed fast dissolution was either by the CyA nano–crystals on the surface of NPs which were too small in size (only a few nm) or due to exposure of these NPs to the PBS medium (pH = 7.4). Moreover, when water diffused in through the pores of CS–CyA NPs, these molecules diffused out and resulted in the fast drug release. While the sustained release might be due to the better crosslinking of CS–CyA NPs mainly due to the presence of TPP (Fig. 13) [148].

The optimization of the crosslinker concentration is one of the important factors which can control the size, shape, zeta potential/stability and drug release properties. In the modification of anti–cancer drug DTX with CS NPs, the TPP was used as a crosslinker and effects of change of its concentration was studied. The particle size significantly decreased as the concentration of TPP increased from 0.25% w/v to 0.75% w/v, whereas, further increase in the TPP concentration resulted in larger particles/agglomeration and low zeta potential. In this case, the optimized concentration of the TPP resulted in compact particles which had control particle size and zeta potential. The synthesized DTX–CS NPs initially exhibited a burst release which could be due to the presence of drug molecules on the surface of NPs, while a sustained release occurred through the polymeric matrix which might be due to the optimized crosslinking with TPP (Fig. 14) [149].

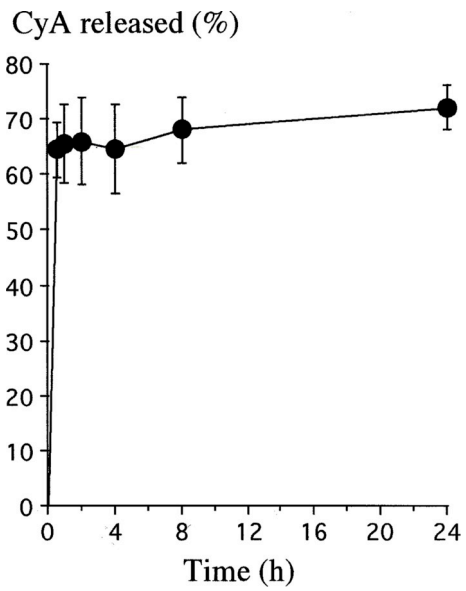

Fig. 13. Release profile of CyA from CS–CyA NPs (crosslinked with TTP = 0.2% w/v) (Reprinted with permission from Ref. [148], Copyright (2001) Elsevier Science B. V.).

The modification of a protein, BSA (1.0 mg/mL) with N–(2–hydroxyl) propyl–3–trimethyl ammonium chitosan chloride (HTCC) NPs by using ionic gelation method resulted in the slow release of BSA in sodium chloride saline (SCS) medium. The use of TPP as a crosslinker enhanced the strength of BSA–CS NPs in the release medium and BSA release was dependent on TPP concentration. A Slow release of BSA was observed in TPP (0.8 mg/mL) crosslinked NPs as compared with TPP (0.5 mg/mL) crossed linked NPs. Likewise previous cases, the burst release (17–45%) was attributed to the adsorption of drug molecules on the particle surfaces while, the slow release was attributed to better encapsulation of BSA due to the TPP crosslinking with HTCC. Somehow the slow release in this case was also improved due to the presence of PEG in the formulation [150]. Similarly, the release of BSA from the water–soluble CS NPs (prepared by ionic gelation) followed three phases; i) burst release due to the desorption of drug from the surface of NPs, (ii), a slow release by diffusion of drug molecules dispersed in the polymeric carrier and (iii), a constant sustained drug release by diffusion from walls of polymeric NPs as a result of erosion (Fig. 15) [151].

The crosslinking of CS with a Tripolyphosphate (TPP) provide better mechanical strength to the CS NPs. This helped the CS NPs to maintain the polymeric matrix for a long time during the drug release study in acidic and basic pH mediums. Some of the drugs are very sensitive that the addition of a certain career material may results in high diffusion rates ultimately resulting in a burst release. Tripolyphosphate (TPP) has the ability to alter the crystallinity of the CS and various drugs providing them the compact structures resulting in less drug diffusion and hence, less drug release. In this regard, the understanding and optimization of the CS-TPP mass ratios, TPP concentration and the initial pH are essential. Each drug has specific interactions with CS, therefore it is necessary to optimize all these parameters (mass ratio, concentration and initial pH) while using TPP as a crosslinker especially for the controlled release applications.

General drug release mechanism through polymeric nanoparticles

The nano drugs are more suitable and advanced than the normal medicines in terms of target capabilities and controlled release [152–154]. The NPs size, hydrophobicity, modified surface and surface

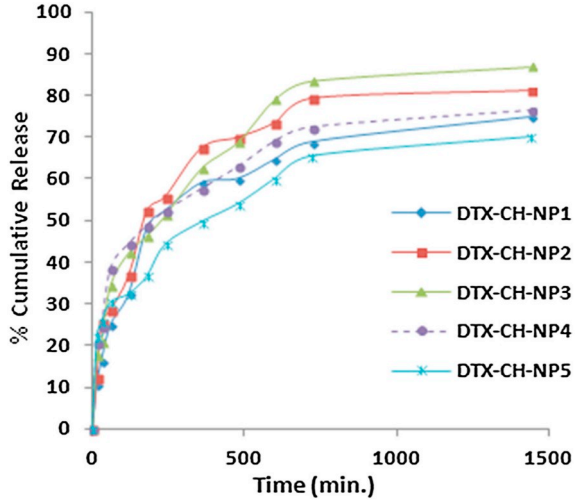

Fig. 14. Release profile of DTX from DTX–CS NPs (Reprinted with permission from Ref. [149], Copyright (2016) Elsevier Ltd.).

charge are the essential factors which control the targeting capabilities [155,156]. The use of NPs in drug delivery applications have various benefits such as enhanced solubility of hydrophobic drugs, controlled and sustained release and improved stability of therapeutic agents [157–159]. The toxicity and side effects of the chemotherapy drugs can be better minimized by using NPs for the TDDS [160]. Drugs can be easily attached to nanocarriers either by adsorption or covalent linkage. Currently, polymers are being widely used for the pharmaceutical applications ranging from general to highly complex DDS. The history shows that the first clinical trials of polymer–drug combination were held during the 1960s, and the successful trials brought forward the multiple potentials of the polymer drugs which are beneficial for the drug delivery applications. The polymeric NPs are considered more advanced than simple NPs due to their capability towards prolonged release with improved drug safety and adjustable pharmacokinetics [161–163]. The drug release mechanism from the polymeric NPs is controlled by the degradation rate of polymer and diffusion of the drug from the polymeric matrix. In this regard, the diffusion of the drug is classified into four basic categories; (i), diffusion of the drug due to water intake, (ii), due to osmotic pressure, (iii), through polymeric matrix, and (iv), due to erosion mechanism. The fact behind the first case is that the polymeric matrix consists of pores and due to biodegradable nature, these pores get wider with time. Water enters the polymeric matrix through these pores and widens, which ultimately make sufficient openings for the drug molecules to diffuse out of the polymeric matrix. In the second case, the drug release occurs through the water–filled pores with a fact that it is convection driven and based on osmotic pumping. In the third case, the drug diffusion is mainly based on the permeability and thickness of polymer [164,165]. In the fourth case, the drug release is based on the rate of degradation of the polymeric matrix. The polymeric material starts degrading from outside to inside and the drug release occurs when the erosion rate is higher than the water penetration rate. This kind of drug release has significance in terms of controllable and reproducible kinetics. In the researcher's opinion, the best way for prediction of the release rate and release mechanisms in drug transportation is to develop an iterative model and compare then with the experimental findings. These modeling techniques can help in highlighting the significance of shape, size, MM), variation of polymer compositions, mass ratios of polymer with crosslinker and type of crosslinker etc over the drug release kinetics. The prediction about the release mechanism can be useful for the optimization of drug release. Till now different models have been used for the better understanding and representation of polymer–drug interaction and also for the determination of release rate of drugs from the polymeric matrix. The most commonly reported kinetic models (zero order, first order and Higuchi) to represent different drug release are shown below:

$$\text{Zero Order:} \cdot D_t = D_0 + k_0\, t \tag{1}$$

$$\text{First order:} \cdot \ln D_t = \ln D_0 + k_1\, t \tag{2}$$

$$\text{Higuchi:} \cdot D_t = D_0 = k_H t^{\frac{1}{2}} \tag{3}$$

where D_t represents the release of drug at time t, D_0 represents the initial release of the drug, k_0, k_1 and k_H represents zero order, first order and Higuchi release constants respectively. Zero order represents the kinetics in which release is independent of drug concentration, while drug release in first order is concentration dependent. The drug release from the Higuchi model is dependent on time. Sometimes route of administration also influences the release kinetics of drugs. For example, most of the drugs which are administered by oral route or through injections exhibit first order release rate, while through the transdermal path exhibit Higuchi release rate. Most of the polymeric nanostructures release the drugs steadily and have the ability to shift the release rate to zero order. The drug release with zero order is considered as a controlled release rate which can significantly provide a safe concentration of the drug. In this way, the side effects can be minimized and extra dosage of drug can be avoided. The zero order kinetics can be achieved with strategic changes such as controlling the morphology (holes). This control over the holes and structure results in limited diffusion of drug through NPs. Similarly, some techniques such as annealing and sonication could improve the release kinetics of drugs (a sustained release of zero order kinetics) [166].

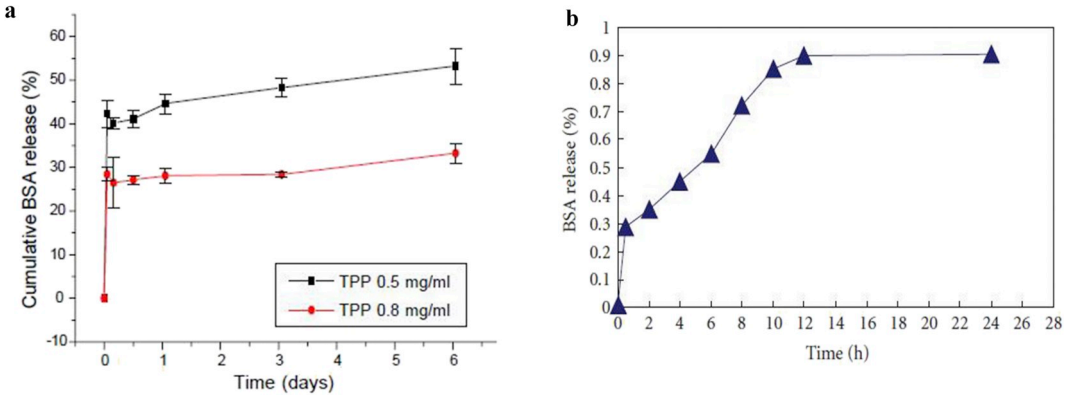

Fig. 15. Release profile of BSA from: (a), HTCC NPs crosslinked with TPP (0.5 mg/mL and 0.8 mg/mL) (Reprinted with permission from Ref. [150], Copyright (2003) Elsevier Ltd.) and (b), BSA–CS NPs crosslinked with TPP (1.0 mg/mL) (Reprinted from Ref. [151], open access Journal Copyright (2010) Hindawi Publishing Corporation).

There is evidence where the size of the particle plays an important role and influence the release rate which means small particles decrease/slow down the release rate. Most of the research reports on the modification of tablets with NPs, revealed that NPs coated tablets possess a slow release of drugs and shifted the release rate to zero order as the size of particles decreased. Similarly, a comparison study between the macroporous carrier and a microporous career for administering the anti–inflammatory drug by oral route revealed that the former resulted in a burst release and then followed by first order release rate, while the microporous carrier resulted in a steady release and then followed by zero order release rate. The polymers exhibit either triphasic or biphasic drug release profiles. The triphasic drug release is divided into three phases out of which the first phase shows a burst/fast release, the second phase shows a slow release, whereas the third phase again showed a fast release. The estimation of 'n' (mechanism of drug release) value classifies the type of the diffusion taking place in drug release profile. If its value is around n = 0.43, it is said to be Fickian type diffusion, while the value n = 0.85 indicates the Case II transport. The value $0.43 < n < 0.85$ is indication of non Fickian type diffusion [167]. The pH of the medium is one of the main components which govern changes over the release profile of drugs. For example, the data of Curcumin release in SGF fits well into the Ritger-Peppas model (equation (4)), where the 'n' indicates that release of Curcumin is dependent on both particles swelling and diffusion (non = Fickian). On the other hand, Curcumin exhibited a burst release from NPs in SIF medium following the first order release kinetics [168].

$$\frac{M_t}{M_\infty} = kt^n \tag{4}$$

where, M_t/M_∞, k and n stand for fractional drug release at time t, Ritger-Peppas constant and drug release mechanism indicator respectively. Another model (Weibull model, equation (5)) can significantly predict both burst and slow release rates. The best fitting of Docetaxel release data from the PLA-CS NPs was obtained by using this model. The kinetics study indicated that drug release in this case followed Fickian diffusion along with polymer erosion mechanism.

$$\ln\left[ln\left(\frac{100}{100-Q}\right)\right] = klnt + b \tag{5}$$

where, Q, k and b stand for the fractional drug release, rate constant and constant respectively. The Hixson-Crowell (equation (6)) is another model based on cube root law which is useful for describing the drug release from the particles when its size and surface area changes.

$$\sqrt[3]{Q_o} - \sqrt[3]{Q_t} = k_{HC}t \tag{6}$$

where Q_o, Q_t, K_{HC} stand for the initial amount of drug, drug amount at time t and rate constant respectively. There are some cases where some of the drugs show the appropriate fitting in multiple kinetic models. The drug (BSA) release from the Carboxymethyl-β-cyclodextrin (CMCD) grafted CS NPs indicated that its release data was fit in various models (zero order, first order and Higuchi). Such kinetic prediction highlighted drug release as a complex process which includes diffusion, erosion and dissolution. The CMCD grafted CS NPs could be a good approach for saving the similar drugs in GIT following a sustained release. In contrast to BSA, insulin release from CMCS-g-CS NPs showed burst release in 15 min [169].

4. Conclusions

Present drug delivery systems which have approval for the clinical use are less efficient to meet the treatment required for a longer time. Due to the short lifespan of several drugs, they diminish in a few minutes and repeated dosages are required. Control over the drug delivery and release system brought the concept of minimizing discomfort and side reactions by reducing the number of dosage for a specific treatment. It is really challenging to keep the concentration of a drug dose between the effective therapeutic levels, such as above the minimum effective concentration and below the maximum toxic concentration of the drug. For this purpose, different carrier materials and routes of administration have been investigated. These carrier materials have been investigated for a number of individual drugs as well as with other possible combinations in the form of microparticles/microspheres/nanoparticles/beads/gels etc. Due to their unique physiochemical properties, some of these materials provided better encapsulation efficiency, while other materials improved the in vitro and in vivo release kinetics of the drugs. With all these benefits, yet most of the modified drugs are still under investigation and need clinical trials.

There are several important factors which need to be considered for the betterment of the drug delivery system. These factors include nature of drug (water solubility and acid/basic sensitivity), nature of carrier material, pH conditions, temperature, the concentration of the carrier material/co–materials and concentration of the cross–linking agent. To bring improvement in the current drug delivery system, it is imperative to investigate the properties of drugs as well as new carrier materials. It is essential to research for new biodegradable materials which are non–toxic to the human body and environment, and can overcome the shortcomings faced by the specific drugs. Optimization of the operating conditions can improve the drug delivery system by improving purity, encapsulation efficiency, stability, toxicity and release profile. The blending of polymers with other natural/synthetic polymers has been a good approach to alter and control the release rate of drugs in acidic and alkaline conditions. A multifunctional complex of NPs is required which can prevent the drug from the harsh conditions and trigger the release of drug at the targeted site for a prolonged time. Among the available polymers, Chitosan (a natural polymer) appeared more attractive to be used for controlled release of drugs and their modifications with new solvents can give a turn over to the drug delivery system especially for the controlled and sustained release of drugs.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jddst.2018.10.020.

References

[1] J. Kost, R. Langer, Responsive polymeric delivery systems, Adv. Drug Deliv. Rev. 64 (2012) 327–341.
[2] A. Kikuchi, T. Okano, Pulsatile drug release control using hydrogels, Adv. Drug Deliv. Rev. 54 (1) (2002) 53–77.
[3] A.A. Gabizon, D. Tzemach, A.T. Horowitz, H. Shmeeda, J. Yeh, S. Zalipsky, Reduced toxicity and superior therapeutic activity of a mitomycin C lipid–based prodrug incorporated in pegylated liposomes, Clin. Canc. Res. 12 (6) (2006) 1913–1920.
[4] J. Baker, J. Ajani, F. Scotte, D. Winther, M. Martin, M.S. Aapro, G. von Minckwitz, Docetaxel–related side effects and their management, Eur. J. Oncol. Nurs. 13 (1) (2009) 49–59.
[5] B.D. Tarr, S.H. Yalkowsky, A new parenteral vehicle for the administration of some poorly soluble anti–cancer drugs, PDA J. Pharm. Sci. Technol. 41 (1987) 31–33.
[6] R.B. Weiss, R.C. Donehower, P.H. Wiernik, Hypersensitivity reactions from taxol, J. Clin. Oncol. 8 (7) (1990) 1263–1268.
[7] C. Puglia, M.R. Lauro, G.G. Tirendi, G.E. Fassari, C. Carbone, F. Bonina, G. Puglisi, Modern drug delivery strategies applied to natural active compounds, Expet Opin. Drug Deliv. 14 (6) (2017) 755–768.
[8] E. Ajorlou, A.Y. Khosroushahi, Trends on polymer–and lipid–based nanostructures for parenteral drug delivery to tumors, Cancer Chemother. Pharmacol. (2017) 1–15.
[9] S. Solari, A. Cancino, R. Wolff, B. Norero, J.I. Vargas, F. Barrera, M. Arrese, Sublingual tacrolimus administration provides similar drug exposure to per-oral route employing lower doses in liver transplantation: a pilot study, Aliment.

Pharmacol. Ther. 45 (9) (2017) 1225–1231.
[10] K. Dutta, D. Hu, B. Zhao, A.E. Ribbe, J. Zhuang, S. Thayumanavan, Templated self–assembly of a covalent polymer network for intracellular protein delivery and traceless release, J. Am. Chem. Soc. 139 (16) (2017) 5676–5679.
[11] T. Pelras, H.T. Duong, B.J. Kim, B.S. Hawkett, M. Müllner, A 'grafting from' approach to polymer nanorods for pH–triggered intracellular drug delivery, Polym 112 (2017) 244–251.
[12] T. Santos, R. Ferreira, J. Maia, F. Agasse, S. Xapelli, L. Cortes, J. Bragança, J.O. Malva, L. Ferreira, L. Bernardino, Polymeric nanoparticles to control the differentiation of neural stem cells in the subventricular zone of the brain, ACS Nano 6 (12) (2012) 10463–10474.
[13] J. Garcia–Salcedo, J. Munday, J. Unciti–Broceta, H. Koning, Progress towards new treatments for human African trypanosomiasis, in: S. Magez, M. Radwanska (Eds.), Trypanosomes and Trypanosomiasis, Springer Vienna, 2014, pp. 217–238.
[14] E.S. Khafagy, M. Morishita, Y. Onuki, K. Takayama, Current challenges in non–-invasive insulin delivery systems: a comparative review, Adv. Drug Deliv. Rev. 59 (15) (2007) 1521–1546.
[15] E.M. Pridgen, F. Alexis, O.C. Farokhzad, Polymeric nanoparticle technologies for oral drug delivery, Clin. Gastroenterol. Hepatol. 12 (10) (2014) 1605–1610.
[16] L. Mei, Z. Zhang, L. Zhao, L. Huang, X.L. Yang, J. Tang, S.S. Feng, Pharmaceutical nanotechnology for oral delivery of anticancer drugs, Adv. Drug Deliv. Rev. 65 (6) (2013) 880–890.
[17] S. Teague, K. Valko, How to identify and eliminate compounds with a risk of high clinical dose during the early phase of lead optimisation in drug discovery, Eur. J. Pharmaceut. Sci. 110 (2017) 37–50.
[18] A. Martin–Vicente, J. Guarro, J. Capilla, (Does a triple combination have better activity than double combinations against multiresistant fungi, Experimental in vitro evaluation, Int. J. Antimicrob. Agents 49 (4) (2017) 422–426.
[19] O. Kayser, C. Olbrich, S.L. Croft, A.F. Kiderlen, Formulation and biopharmaceutical issues in the development of drug delivery systems for antiparasitic drugs, Parasitol. Res. 90 (2) (2003) S63–S70.
[20] S. Mitragotri, J. Lahann, Materials for drug delivery: innovative solutions to address complex biological hurdles, Adv. Mater. 24 (2012) 3717–3723.
[21] S.A. Papadimitriou, Y. Salinas, M. Resmini, Smart polymeric nanoparticles as emerging tools for imaging—the parallel evolution of materials, Chem. Eur J. 22 (11) (2016) 3612–3620.
[22] S. Mura, J. Nicolas, P. Couvreur, Stimuli–responsive nanocarriers for drug delivery, Nat. Mater. 12 (11) (2013) 991–1003.
[23] E. Ajorlou, A.Y. Khosroushahi, Trends on polymer–and lipid–based nanostructures for parenteral drug delivery to tumors, Cancer Chemother. Pharmacol. (2017) 1–15.
[24] S.J. Lam, E.H. Wong, C. Boyer, G.G. Qiao, Antimicrobial polymeric nanoparticles, Prog. Polym. Sci. 76 (2018) 40–64.
[25] F.S. Kittur, A.B.V. Kumar, R.N. Tharanathan, Low molecular weight chitosans—preparation by depolymerization with Aspergillus Niger pectinase, and characterization, Carbohydr. Res. 338 (12) (2003) 1283–1290.
[26] S.A. Agnihotri, N.N. Mallikarjuna, T.M. Aminabhavi, Recent advances on chitosan–based micro–and nanoparticles in drug delivery, J. Contr. Release 100 (1) (2004) 5–28.
[27] B. Krajewska, Membrane–based processes performed with use of chitin/chitosan materials, Separ. Purif. Technol. 41 (3) (2005) 305–312.
[28] P. Sahariah, M. Masson, Antimicrobial chitosan and chitosan derivatives: a review of the structure–activity relationship, Biomacromolecules 18 (11) (2017) 3846–3868.
[29] C. Liu, G. Wang, W. Sui, L. An, C. Si, Preparation and characterization of chitosan by a novel deacetylation approach using glycerol as green reaction solvent, ACS Sustain. Chem. Eng. 5 (6) (2017) 4690–4698.
[30] E. Vunain, A.K. Mishra, B.B. Mamba, Fundamentals of chitosan for biomedical applications, In Chitosan Based Biomaterials 1 (2017) 3–30.
[31] M. Garcia-Fuentes, M.J. Alonso, Chitosan-based drug nanocarriers: where do we stand, J. Contr. Release 161 (2) (2012) 496–504.
[32] J. Liu, H. Pu, S. Liu, J. Kan, C. Jin, Synthesis, characterization, bioactivity and potential application of phenolic acid grafted chitosan: a review, Carbohydr. Polym. 174 (2017) 999–1017.
[33] N.G. Schipper, S. Olsson, J.A. Hoogstraate, K.M. Vårum, P. Artursson, Chitosans as absorption enhancers for poorly absorbable drugs 2: mechanism of absorption enhancement, Pharm. Res. 14 (7) (1997) 923–929.
[34] L. Casettari, L. Illum, Chitosan in nasal delivery systems for therapeutic drugs, J. Contr. Release 190 (2014) 189–200.
[35] B.E. Benediktsdóttir, Ó. Baldursson, M. Másson, Challenges in evaluation of chitosan and trimethylated chitosan (TMC) as mucosal permeation enhancers: from synthesis to in vitro application, J. Contr. Release 173 (2014) 18–31.
[36] S. Papadimitriou, D. Bikiaris, K. Avgoustakis, E. Karavas, M. Georgarakis, Chitosan nanoparticles loaded with dorzolamide and pramipexole, Carbohydr. Polym. 73 (1) (2008) 44–54.
[37] M.A. Elgadir, M.S. Uddin, S. Ferdosh, A. Adam, A.J.K. Chowdhury, M.Z.I. Sarker, Impact of chitosan composites and chitosan nanoparticle composites on various drug delivery systems: a review, J. Food Drug Anal. 23 (4) (2015) 619–629.
[38] J. Malmo, K.M. Vårum, S.P. Strand, Effect of chitosan chain architecture on gene delivery: comparison of self–branched and linear chitosans, Biomacromolecules 12 (3) (2011) 721–729.
[39] M. Kong, X.G. Chen, K. Xing, H.J. Park, Antimicrobial properties of chitosan and mode of action: a state of the art review, Int. J. Food Microbiol. 144 (1) (2010) 51–63.
[40] M. Ahmad, K. Manzoor, S. Singh, S. Ikram, Chitosan centered bionanocomposites for medical specialty and curative applications: a review, Int. J. Pharm. 529 (1–2) (2017) 200–217.
[41] Q. Gan, T. Wang, Chitosan nanoparticle as protein delivery Carrier—systematic examination of fabrication conditions for efficient loading and release, Colloids Surf., B 59 (1) (2007) 24–34.
[42] A. Bernkop-Schnürch, S. Dünnhaupt, Chitosan-based drug delivery systems, Eur. J. Pharm. Biopharm. 81 (3) (2012) 463–469.
[43] Y. Boonsongrit, B.W. Mueller, A. Mitrevej, Characterization of drug–chitosan interaction by 1H NMR, FTIR and isothermal titration calorimetry, Eur. J. Pharm. Biopharm. 69 (1) (2008) 388–395.
[44] P. Calvo, C. Remuñan-López, J.L. Vila-Jato, M.J. Alonso, Chitosan and chitosan/ethylene oxide-propylene oxide block copolymer nanoparticles as novel carriers for proteins and vaccines, Pharm. Res. 14 (10) (1997) 1431–1436.
[45] Y. Boonsongrit, A. Mitrevej, B.W. Mueller, Chitosan drug binding by ionic interaction, Eur. J. Pharm. Biopharm. 62 (3) (2006) 267–274.
[46] Y. Luo, Q. Wang, Recent development of chitosan-based polyelectrolyte complexes with natural polysaccharides for drug delivery, Int. J. Biol. Macromol. 64 (2014) 353–367.
[47] G. Lawrie I. Keen, B. Drew, A. Chandler-Temple, L. Rintoul, P. Fredericks, L. Grøndahl, Interactions between alginate and chitosan biopolymers characterized using FTIR and XPS, Biomacromolecules 8 (8) (2007) 2533–2541.
[48] R.K. Katarina, T. Takayanagi, M. Oshima, S. Motomizu, Synthesis of a chitosan–based chelating resin and its application to the selective concentration and ultratrace determination of silver in environmental water samples, Anal. Chim. Acta 558 (1) (2006) 246–253.
[49] S. Zhang, J. Feng, J. Feng, Y. Jiang, Oxidation–mediated chitosan as additives for creation of chitosan aerogels with diverse three–dimensional interconnected skeletons, Appl. Surf. Sci. 396 (2017) 1220–1225.
[50] Y. Xu, Y. Du, Effect of molecular structure of chitosan on protein delivery properties of chitosan nanoparticles, Int. J. Pharm. 250 (1) (2003) 215–226.
[51] C.R. Huei, H.D. Hwa, Effect of molecular weight of chitosan with the same degree of deacetylation on the thermal, mechanical, and permeability properties of the prepared membrane, Carbohydr. Polym. 29 (4) (1996) 353–358.
[52] M.R. Kasaai, J. Arul, G. Charlet, Intrinsic viscosity–molecular weight relationship for chitosan, J. Polym. Sci., Polym. Phys. Ed. 38 (19) (2000) 2591–2598.
[53] S. Kume, H. Kobayashi, Molecular weight measurement by vapor pressure osmometer, Macromol. Chem. Phys. 79 (1) (1964) 1–7.
[54] S.A. Umoren, M.M. Solomon, Polymer characterization: polymer molecular weight determination, Polymer Science: Research Advances, practical applications and educational aspects (2016) 412–419.
[55] T.F. Page, W.E. Bresler, End-group Analysis and number-average molecular weight determination of some polyalkylene glycols and glycol polyesters using nuclear magnetic resonance spectroscopy, Anal. Chem. 36 (10) (1964) 1981–1985.
[56] J. Liu, R.S. Loewe, R.D. McCullough, Employing MALDI-MS on poly (alkylthiophenes): analysis of molecular weights, molecular weight distributions, end-group structures, and end-group modifications, Macromolecules 32 (18) (1999) 5777–5785.
[57] G. Paradossi, D.A. Brant, Light scattering study of a series of xanthan fractions in aqueous solution, Macromolecules 15 (3) (1982) 874–879.
[58] R.L. Cleland, J.L. Wang, Ionic polysaccharides. III. Dilute solution properties of hyaluronic acid fractions, Biopolymers 9 (7) (1970) 799–810.
[59] P. Debye, Molecular-weight determination by light scattering, J. Phys. Chem. 51 (1) (1947) 18–32.
[60] S. Nguyen, F.M. Winnik, M.D. Buschmann, Improved reproducibility in the determination of the molecular weight of chitosan by analytical size exclusion chromatography, Carbohydr. Polym. 75 (3) (2009) 528–533.
[61] A. Fiamingo, J.A. de Moura Delezuk, S. Trombotto, L. David, S.P. Campana-Filho, Extensively deacetylated high molecular weight chitosan from the multistep ultrasound-assisted deacetylation of beta-chitin, Ultrason. Sonochem. 32 (2016) 79–85.
[62] M.G. Neira-Velázquez, M.T. Rodríguez-Hernández, E. Hernández-Hernández, A.R. Ruiz-Martínez, Polymer molecular weight measurement, handbook of polymer synthesis, Characterization, and Processing (2013) 355–366.
[63] J.C. Moore, J.G. Hendrickson, Gel permeation chromatography. II, the nature of the separation, J. Polym. Sci. 8 (1) (1965) 233–241.
[64] N. Yacob, N. Talip, M. Mahmud, N.A.I.M. Sani, N.A. Samsuddin, N.A. Fabillah, Determination of Viscosity-average Molecular Weight of Chitosan Using Intrinsic Viscosity Measurement, NTC Nuclear Technical Convention 2011, Malaysia, 2011.
[65] W. Boonyo, H.E. Junginger, N. Waranuch, A. Polnok, T. Pitaksuteepong, Preparation and characterization of particles from chitosan with different molecular weights and their trimethyl chitosan derivatives for nasal immunization, J. Met. Mater. Min. 18 (2) (2017).
[66] Z. Sun, C. Shi, X. Wang, Q. Fang, J. Huang, Synthesis, characterization, and antimicrobial activities of sulfonated chitosan, Carbohydr. Polym. 155 (2017) 321–328.
[67] I. Dueramae, M. Yoneyama, N. Shinyashiki, S. Yagihara, R. Kita, Self-assembly of acetylated dextran with various acetylation degrees in aqueous solutions: studied by light scattering, Carbohydr. Polym. 159 (2017) 171–177.
[68] T. Ito, T. Takami, Y. Uchida, Y. Murakami, Chitosan gel sheet containing drug carriers with controllable drug-release properties, Colloids. Surf. B 163 (2018) 257–265.
[69] C. Zheng, H. Gao, D.P. Yang, M. Liu, H. Cheng, Y.L. Wu, X.J. Loh, PCL-based thermo-gelling polymers for in vivo delivery of chemotherapeutics to tumors, Mater. Sci. Eng. C 74 (2017) 110–116.
[70] C.P. Silva, J.H. Martínez, K.D. Martínez, M.E. Farías, F.C. Leskow, O.E. Pérez, Proposed molecular model for electrostatic interactions between insulin and chitosan. Nano-complexation and activity in cultured cells, Colloids. Surf. A

Physicochem. Eng. Aspects 537 (2018) 425–434.
[71] C.F. João, A.T. Kullberg, J.C. Silva, J.P. Borges, Chitosan Inverted Colloidal Crystal scaffolds: influence of molecular weight on structural stability, Mater. Lett. 193 (2017) 50–53.
[72] S. Kadouche, M. Farhat, H. Lounici, M. Fiallo, P. Sharrock, M. Mecherri, M. Hadioui, Low cost chitosan biopolymer for environmental use made from abundant shrimp wastes, Waste Biomass Valor 8 (2) (2017) 401–406.
[73] Y. Wang, B. Li, X. Zhang, N. Peng, Y. Mei, Y. Liang, Low molecular weight chitosan is an effective antifungal agent against Botryosphaeria sp. and preservative agent for pear (Pyrus) fruits, Int. J. Biol. Macromol. 95 (2017) 1135–1143.
[74] I. Younes, S. Sellimi, M. Rinaudo, K. Jellouli, M. Nasri, Influence of acetylation degree and molecular weight of homogeneous chitosans on antibacterial and antifungal activities, Int. J. Food Microbiol. 185 (2014) 57–63.
[75] S. Viel, D. Capitani, L. Mannina, A. Segre, Diffusion-ordered NMR spectroscopy: a versatile tool for the molecular weight determination of uncharged polysaccharides, Biomacromolecules 4 (6) (2003) 1843–1847.
[76] S. Iqbal, R. Marchetti, A. Aman, A. Silipo, S.A.U. Qader, A. Molinaro, Enzymatic and acidic degradation of high molecular weight dextran into low molecular weight and its characterizations using novel Diffusion-ordered NMR spectroscopy, Int. J. Biol. Macromol. 103 (2017) 744–750.
[77] Q.Z. Wang, X.G. Chen, N. Liu, S.X. Wang, C.S. Liu, X.H. Meng, C.G. Liu, Protonation constants of chitosan with different molecular weight and degree of deacetylation, Carbohydr. Polym. 65 (2) (2006) 194–201.
[78] C. Feng, Z. Wang, C. Jiang, M. Kong, X. Zhou, Y. Li, X. Chen, Chitosan/o–carboxymethyl chitosan nanoparticles for efficient and safe oral anticancer drug delivery: in vitro and in vivo evaluation, Int. J. Pharm. 457 (1) (2013) 158–167.
[79] L. Bekale, D. Agudelo, H.A. Tajmir–Riahi, Effect of polymer molecular weight on chitosan–protein interaction, Colloids Surf., B 125 (2015) 309–317.
[80] S.A. Papadimitriou, D.S. Achilias, D.N. Bikiaris, Chitosan-g-PEG nanoparticles ionically crosslinked with poly (glutamic acid) and tripolyphosphate as protein delivery systems, Int. J. Pharm. 430 (1–2) (2012) 318–327.
[81] Y. Wu, W. Yang, C. Wang, J. Hu, S. Fu, Chitosan nanoparticles as a novel delivery system for ammonium glycyrrhizinate, Int. J. Pharm. 295 (1) (2005) 235–245.
[82] M. Prabaharan, Chitosan–based nanoparticles for tumor–targeted drug delivery, Int. J. Biol. Macromol. 72 (2015) 1313–1322.
[83] M. Zhang, X.H. Li, Y.D. Gong, N.M. Zhao, X.F. Zhang, Properties and biocompatibility of chitosan films modified by blending with PEG, Biomaterials 23 (13) (2002) 2641–2648.
[84] M. Bernela, M. Ahuja, R. Thakur, Enhancement of anti–inflammatory activity of glycyrrhizic acid by encapsulation in chitosan–katira gum nanoparticles, Eur. J. Pharm. Biopharm. 105 (2016) 141–147.
[85] Q. Gan, T. Wang, C. Cochrane, P. McCarron, Modulation of surface charge, particle size and morphological properties of chitosan–TPP nanoparticles intended for gene delivery, Colloids Surf., B 44 (2–3) (2005) 65–73.
[86] M.L. Tsai, R.H. Chen, S.W. Bai, W.Y. Chen, The storage stability of chitosan/tripolyphosphate nanoparticles in a phosphate buffer, Carbohydr. Polym. 84 (2) (2011) 756–761.
[87] J. Antoniou, F. Liu, H. Majeed, J. Qi, W. Yokoyama, F. Zhong, Physicochemical and morphological properties of size-controlled chitosan–tripolyphosphate nanoparticles, Colloids Surf. A Physicochem. Eng. Aspects 465 (2015) 137–146.
[88] D.D. Chinta, R.A. Graves, S. Pamujula, N. Praetorius, L.A. Bostanian, T.K. Mandal, Spray–dried chitosan as a direct compression tableting excipients, Drug Dev. Ind. Pharm. 35 (1) (2009) 43–48.
[89] R. Khlibsuwan, T. Pongjanyakul, Chitosan–clay matrix tablets for sustained–release drug delivery: effect of chitosan molecular weight and lubricant, J. Drug Deliv. Sci. Technol. 35 (2016) 303–313.
[90] K. Huanbutta, P. Sriamornsak, S. Limmatvapirat, M. Luangtana–anan, Y. Yoshihashi, E. Yonemochi, J. Nunthanid, Swelling kinetics of spray–dried chitosan acetate assessed by magnetic resonance imaging and their relation to drug release kinetics of chitosan matrix tablets, Eur. J. Pharm. Biopharm. 77 (2) (2011) 320–326.
[91] V. Bansal, P.K. Sharma, N. Sharma, O.P. Pal, R. Malviya, Applications of chitosan and chitosan derivatives in drug delivery, Adv. Biol. Res. 5 (1) (2011) 28–37.
[92] L. Qi, Z. Xu, X. Jiang, C. Hu, X. Zou, Preparation and antibacterial activity of chitosan nanoparticles, Carbohydr. Res. 339 (16) (2004) 2693–2700.
[93] Z.M. Dos Santos, A.L.P.F. Caroni, M.R. Pereira, D.R. Da Silva, J.L.C. Fonseca, Determination of deacetylation degree of chitosan: a comparison between conductometric titration and CHN elemental analysis, Carbohydr. Res. 344 (18) (2009) 2591–2595.
[94] Y. Zhang, X. Zhang, R. Ding, J. Zhang, J. Liu, Determination of the degree of deacetylation of chitosan by potentiometric titration preceded by enzymatic pretreatment, Carbohydr. Polym 83 (2) (2011) 813–817.
[95] X. Jiang, L. Chen, W. Zhong, A new linear potentiometric titration method for the determination of deacetylation degree of chitosan, Carbohydr. Polym. 54 (4) (2003) 457–463.
[96] M.R. Kasaai, Various methods for determination of the degree of N-acetylation of chitin and chitosan: a review, J. Agric. Food Chem. 57 (5) (2009) 1667–1676.
[97] E. Fernandez-Megia, R. Novoa-Carballal, E. Quiñoá, R. Riguera, Optimal routine conditions for the determination of the degree of acetylation of chitosan by 1H-NMR, Carbohydr. Polym. 61 (2) (2005) 155–161.
[98] L. Heux, J. Brugnerotto, J. Desbrieres, M.F. Versali, M. Rinaudo, Solid state NMR for determination of degree of acetylation of chitin and chitosan, Biomacromolecules 1 (4) (2000) 746–751.
[99] N. Balázs, P. Sipos, Limitations of pH-potentiometric titration for the determination of the degree of deacetylation of chitosan, Carbohydr. Res. 342 (1) (2007) 124–130.
[100] T. Hattori, K. Katai, M. Kato, M. Izume, Y. Mizuta, Colloidal titration of chitosan and critical unit of chitosan to the potentiometric colloidal titration with poly (vinyl sulfate) using toluidine blue as indicator, Bull. Chem. Soc. Jpn. 72 (1) (1999) 37–41.
[101] E.S. de Alvarenga, C.P. de Oliveira, C.R. Bellato, An approach to understanding the deacetylation degree of chitosan, Carbohydr. Polym. 80 (4) (2010) 1155–1160.
[102] F. Niola, N. Basora, E. Chornet, P.F. Vidal, A rapid method for the determination of the degree of N-acetylation of chitin-chitosan samples by acid hydrolysis and HPLC, Carbohydr. Res. 238 (1993) 1–9.
[103] X. Yan, H.M. Evenocheck, Chitosan analysis using acid hydrolysis and HPLC/UV, Carbohydr. Polym. 87 (2) (2012) 1774–1778.
[104] Z. Han, Y. Zeng, H. Lu, L. Zhang, Determination of the degree of acetylation and the distribution of acetyl groups in chitosan by HPLC analysis of nitrous acid degraded and PMP labeled products, Carbohydr. Res. 413 (2015) 75–84.
[105] H. Sato, S.I. Mizutani, S. Tsuge, H. Ohtani, K. Aoi, A. Takasu, M. Okada, S. Kobayashi, T. Kiyosada, S.I. Shoda, Determination of the degree of acetylation of chitin/chitosan by pyrolysis-gas chromatography in the presence of oxalic acid, Anal. Chem. 70 (1) (1998) 7–12.
[106] A. Hirai, H. Odani, A. Nakajima, Determination of degree of deacetylation of chitosan by 1 H NMR spectroscopy, Polym. Bull. 26 (1) (1991) 87–94.
[107] M. Lavertu, Z. Xia, A.N. Serreqi, M. Berrada, A. Rodrigues, D. Wang, M.D. Buschmann, A. Gupta, A validated 1H NMR method for the determination of the degree of deacetylation of chitosan, J. Pharmaceut. Biomed. Anal. 32 (6) (2003) 1149–1158.
[108] C. Chaouat, S. Balayssac, M. Malet-Martino, F. Belaubre, E. Questel, A.M. Schmitt, S. Poigny, S. Franceschi, E. Perez, Green microparticles based on a chitosan/lactobionic acid/linoleic acid association. Characterisation and evaluation as a new Carrier system for cosmetics, J. Microencapsul. 34 (2) (2017) 162–170.
[109] C. King, R.S. Stein, J.L. Shamshina, R.D. Rogers, Measuring the purity of chitin with a clean, quantitative solid-state NMR method, ACS Sustain. Chem. Eng. 5 (9) (2017) 8011–8016.
[110] R.Y. Zhang, E. Zaslavski, G. Vasilyev, M. Boas, E. Zussman, Tunable pH-responsive Chitosan-Poly (acrylic acid) electrospun fibers, Biomacromolecules 19 (2) (2018) 588–595.
[111] P.L. Ma, M. Lavertu, F.M. Winnik, M.D. Buschmann, Stability and binding affinity of DNA/chitosan complexes by polyanion competition, Carbohydr. Polym. 176 (2017) 167–176.
[112] P. Taşkın, H. Canısağ, M. Sen, The effect of degree of deacetylation on the radiation induced degradation of chitosan, Radiat. Phys. Chem. 94 (2014) 236–239.
[113] H.C. Yang, M.H. Hon, The effect of the degree of deacetylation of chitosan nanoparticles and its characterization and encapsulation efficiency on drug delivery, Polym. Technol. Eng. 49 (12) (2010) 1292–1296.
[114] H. Koo, K.H. Min, S.C. Lee, J.H. Park, K. Park, S.Y. Jeong, K. Kim, Enhanced drug–loading and therapeutic efficacy of hydrotropic oligomer–conjugated glycol chitosan nanoparticles for tumor–targeted paclitaxel delivery, J. Contr. Release 172 (3) (2013) 823–831.
[115] W. Fan, W. Yan, Z. Xu, H. Ni, Formation mechanism of monodisperse, low molecular weight chitosan nanoparticles by ionic gelation technique, Colloids, Surf. B 90 (2012) 21–27.
[116] X.Z. Shu, K.J. Zhu, The influence of multivalent phosphate structure on the properties of ionically cross-linked chitosan films for controlled drug release, Eur. J. Pharm. Biopharm. 54 (2) (2002) 235–243.
[117] B. Hu, C. Pan, Y. Sun, Z. Hou, H. Ye, B. Hu, X. Zeng, Optimization of fabrication parameters to produce chitosan− tripolyphosphate nanoparticles for delivery of tea catechins, J. Agric. Food Chem. 56 (16) (2008) 7451–7458.
[118] A. Rampino, M. Borgogna, P. Blasi, B. Bellich, A. Cesàro, Chitosan nanoparticles: preparation, size evolution and stability, Int. J. Pharm. 455 (1–2) (2013) 219–228.
[119] F. Rázga, D. Vnuková, V. Némethová, P. Mazancová, I. Lacík, Preparation of chitosan-TPP sub-micron particles: critical evaluation and derived recommendations, Carbohydr. Polym. 151 (2016) 488–499.
[120] C.C. Sipoli, N. Santana, A.A.M. Shimojo, A. Azzoni, L.G. de la Torre, Scalable production of highly concentrated chitosan/TPP nanoparticles in different pHs and evaluation of the in vitro transfection efficiency, Biochem. Eng. J. 94 (2015) 65–73.
[121] D.R. Bhumkar, V.B. Pokharkar, Studies on effect of pH on cross–linking of chitosan with sodium tripolyphosphate: a technical note, Aaps PharmSciTech 7 (2) (2006) E138–E143.
[122] L. Zhang, Z.L. Zhao, X.H. Wei, J.H. Liu, Preparation and in vitro and in vivo characterization of cyclosporin A–loaded, PEGylated chitosan–modified, lipid–based nanoparticles, Int. J. Nanomed. 8 (2013) 601.
[123] P. Tang, H. Yang, B. Tang, D. Wu, Q. Du, K. Xu, H. Li, Dimethyl-β-cyclodextrin/salazosulfapyridine inclusion complex-loaded chitosan nanoparticles for sustained release, Carbohydr. Polym. 156 (2017) 215–222.
[124] C. Chen, Y. Yu, X. Wang, P. Shi, Y. Wang, P. Wang, Manipulation of pH–Sensitive interactions between podophyllotoxin–chitosan for enhanced controlled drug release, Int. J. Biol. Macromol. 95 (2017) 451–461.
[125] A. Bernkop–Schnürch, S. Dünnhaupt, Chitosan–based drug delivery systems, Eur. J. Pharm. Biopharm. 81 (3) (2012) 463–469.
[126] B. Fonseca–Santos, M. Chorilli, An overview of carboxymethyl derivatives of chitosan: their use as biomaterials and drug delivery systems, Mater. Sci. Eng. 77 (2017) 1349–1362.
[127] A. Anitha, S. Maya, N. Deepa, K.P. Chennazhi, S.V. Nair, R. Jayakumar, Curcumin–loaded N, O–carboxymethyl chitosan nanoparticles for cancer drug delivery, J. Biomater. Sci. Polym. Ed. 23 (11) (2012) 1381–1400.
[128] X. Bai, M. Kong, G. Xia, S. Bi, Z. Zhou, C. Feng, X. Cheng, X. Chen, Systematic investigation of fabrication conditions of nanocarrier based on carboxymethyl

chitosan for sustained release of insulin, Int. J. Biol. Macromol. 102 (2017) 468–474.
[129] E. Zhang, R. Xing, S. Liu, K. Li, Y. Qin, H. Yu, P. Li, Comparison in docetaxel-loaded nanoparticles based on three different carboxymethyl chitosans, Int. J. Biol. Macromol. 101 (2017) 1012–1018.
[130] N. Ahmadi Nasab, H. Hassani Kumleh, M. Beygzadeh, S. Teimourian, M. Kazemzad, Delivery of curcumin by a pH–responsive chitosan mesoporous silica nanoparticles for cancer treatment, Artif. Cells Nanomed. Biotechnol. (2017) 75–81.
[131] S. Esfandiarpour–Boroujeni, S. Bagheri–Khoulenjani, H. Mirzadeh, S. Amanpour, Fabrication and study of curcumin loaded nanoparticles based on folate–chitosan for breast cancer therapy application, Carbohydr. Polym. 168 (2017) 14–21.
[132] H. Yu, M.H. Nguyen, K. Hadinoto, Effects of chitosan molecular weight on the physical and dissolution characteristics of amorphous curcumin–chitosan nanoparticle complex, Drug Dev. Ind. Pharm. (2017) 1–7.
[133] Q. Yuan, J. Shah, S. Hein, R.D.K. Misra, Controlled and extended drug release behavior of chitosan–based nanoparticle Carrier, Acta Biomater. 6 (3) (2010) 1140–1148.
[134] X. Wang, B. Wei, X. Cheng, J. Wang, R. Tang, 3–Carboxyphenylboronic acid–-modified carboxymethyl chitosan nanoparticles for improved tumor targeting and inhibitory, Eur. J. Pharm. Biopharm. 113 (2017) 168–177.
[135] H. Yang, D.H. Bremner, L. Tao, H. Li, J. Hu, L. Zhu, Carboxymethyl chitosan-mediated synthesis of hyaluronic acid-targeted graphene oxide for cancer drug delivery, Carbohydr. Polym. 135 (2016) 72–78.
[136] L. Zhang, J. Wang, C. Ni, Y. Zhang, G. Shi, Preparation of polyelectrolyte complex nanoparticles of chitosan and poly (2–acry1amido–2–methylpropanesulfonic acid) for doxorubicin release, Mater. Sci. Eng. C 58 (2016) 724–729.
[137] W.J. Trickler, A.A. Nagvekar, A.K. Dash, A novel nanoparticle formulation for sustained paclitaxel delivery, AAPS PharmSciTech 9 (2) (2008) 486–493.
[138] Y. Chen, V.J. Mohanraj, J.E. Parkin, Chitosan-dextran sulfate nanoparticles for delivery of an anti-angiogenesis peptide, Lett. Pept. Sci. 10 (2004) 621–629.
[139] T. Sakiyama, H. Takata, T. Toga, K. Nakanishi, pH-sensitive shrinking of a dextran sulfate/chitosan complex gel and its promotion effect on the release of polymeric substances, J. Appl. Polym. Sci. 81 (2001) 667–674.
[140] B. Sarmento, A. Ribeiro, F. Veiga, D. Ferreira, R. Neufeld, Oral bioavailability of insulin contained in polysaccharide nanoparticles, Biomacromolecules 8 (10) (2007) 3054–3060.
[141] L. Bugnicourt, C. Ladavière, Interests of chitosan nanoparticles ionically cross-linked with tripolyphosphate for biomedical applications, Prog. Polym. Sci. 60 (2016) 1–17.
[142] S.F. Hosseini, M.R. Soleimani, M. Nikkhah, Chitosan/sodium tripolyphosphate nanoparticles as efficient vehicles for antioxidant peptidic fraction from common kilka, Int. J. Biol. Macromol. 111 (2018) 730–737.
[143] J. Wu, Y. Wang, H. Yang, X. Liu, Z. Lu, Preparation and biological activity studies of resveratrol loaded ionically cross-linked chitosan-TPP nanoparticles, Carbohydr. Polym. 175 (2017) 170–177.
[144] I. Dmour, M.O. Taha, Novel nanoparticles based on chitosan-dicarboxylate conjugates via tandem ionotropic/covalent crosslinking with tripolyphosphate and subsequent evaluation as drug delivery vehicles, Int. J. Pharm. 529 (1–2) (2017) 15–31.
[145] E.N. Koukaras, S.A. Papadimitriou, D.N. Bikiaris, G.E. Froudakis, Insight on the formation of chitosan nanoparticles through ionotropic gelation with tripolyphosphate, Mol. Pharm. 9 (10) (2012) 2856–2862.
[146] C. Prego, P. Paolicelli, B. Díaz, S. Vicente, A. Sánchez, Á. González-Fernández, M.J. Alonso, Chitosan-based nanoparticles for improving immunization against hepatitis B infection, Vaccine 28 (14) (2010) 2607–2614.
[147] P.M. Oliveira, B.N. Matos, P.A. Pereira, T. Gratieri, L.H. Faccioli, M.S. Cunha–Filho, G.M. Gelfuso, Microparticles prepared with 50–190 kDa chitosan as promising non–toxic carriers for pulmonary delivery of isoniazid, Carbohydr. Polym. 174 (2017) 421–431.
[148] A.M. De Campos, A. Sánchez, M.J. Alonso, Chitosan nanoparticles: a new vehicle for the improvement of the delivery of drugs to the ocular surface, Application to cyclosporin A, Int. J. Pharm. 224 (1) (2001) 159–168.
[149] A. Jain, K. Thakur, G. Sharma, P. Kush, U.K. Jain, Fabrication, characterization and cytotoxicity studies of ionically cross–linked docetaxel loaded chitosan nanoparticles, Carbohydr. Polym. 137 (2016) 65–74.
[150] Y. Xu, Y. Du, R. Huang, L. Gao, Preparation and modification of N-(2-hydroxyl) propyl-3-trimethyl ammonium chitosan chloride nanoparticle as a protein Carrier, Biomaterials 24 (27) (2003) 5015–5022.
[151] H.L. Zhang, S.H. Wu, Y. Tao, L.Q. Zang, Z.Q. Su, Preparation and characterization of water–soluble chitosan nanoparticles as protein delivery system, J. Nanomater. 2010 (2010) 1.
[152] T. Ramasamy, H.B. Ruttala, B. Gupta, B.K. Poudel, H.G. Choi, C.S. Yong, J.O. Kim, Smart chemistry-based nanosized drug delivery systems for systemic applications: a comprehensive review, J. Contr. Release 258 (2017) 226–253.
[153] C. Cánepa, J.C. Imperiale, C.A. Berini, M. Lewicki, A. Sosnik, M.M. Biglione, Development of a drug delivery system based on chitosan nanoparticles for oral administration of interferon-α, Biomacromolecules 18 (10) (2017) 3302–3309.
[154] J. Wang, J.A. Kaplan, Y.L. Colson, M.W. Grinstaff, Mechanoresponsive materials for drug delivery: harnessing forces for controlled release, Adv. Drug Deliv. Rev. 108 (2017) 68–82.
[155] A.Z. Wilczewska, K. Niemirowicz, K.H. Markiewicz, H. Car, Nanoparticles as drug delivery systems, Pharmacol, For. Rep. 64 (5) (2012) 1020–1037.
[156] U. Wais, A.W. Jackson, Y. Zuo, Y. Xiang, T. He, H. Zhang, Drug nanoparticles by emulsion–freeze–drying via the employment of branched block copolymer nanoparticles, J. Contr. Release 222 (2016) 141–150.
[157] R. Goyal, L.K. Macri, H.M. Kaplan, J. Kohn, Nanoparticles and nanofibers for topical drug delivery, J. Contr. Release 240 (2016) 77–92.
[158] K. Cho, X.U. Wang, S. Nie, D.M. Shin, Therapeutic nanoparticles for drug delivery in cancer, Clin. Canc. Res. 14 (5) (2008) 1310–1316.
[159] Z. Chen, L. Zhang, Y. Song, J. He, L. Wu, C. Zhao, W. Li, Hierarchical targeted hepatocyte mitochondrial multifunctional chitosan nanoparticles for anticancer drug delivery, Biomaterials 52 (2015) 240–250.
[160] J. Li, F. Yu, Y. Chen, D. Oupický, Polymeric drugs: advances in the development of pharmacologically active polymers, J. Contr. Release 219 (2015) 369–382.
[161] L.E. Van Vlerken, T.K. Vyas, M.M. Amiji, Poly (ethylene glycol)–modified nanocarriers for tumor–targeted and intracellular delivery, Pharm. Res. 24 (8) (2007) 1405–1414.
[162] D.B. Shenoy, M.M. Amiji, Poly (ethylene oxide)–modified poly (ε–caprolactone) nanoparticles for targeted delivery of tamoxifen in breast cancer, Int. J. Pharm. 293 (1) (2005) 261–270.
[163] M. Beck–Broichsitter, O.M. Merkel, T. Kissel, Controlled pulmonary drug and gene delivery using polymeric nano–carriers, J. Contr. Release 161 (2) (2012) 214–224.
[164] R.A. Keraliya, C. Patel, P. Patel, V. Keraliya, T.G. Soni, R.C. Patel, M.M. Patel, Osmotic drug delivery system as a part of modified release dosage form, ISRN Pharm. 2012 (2012).
[165] Y. Fu, W.J. Kao, Drug release kinetics and transport mechanisms of non–degradable and degradable polymeric delivery systems, Expert Opin, Drug Deliv. 7 (4) (2010) 429–444.
[166] M.S. Birajdar, J. Lee, Sonication-triggered zero-order release by uncorking core–shell nanofibers, Chem. Eng. J. 288 (2016) 1–8.
[167] J.M. Unagollaand, A.C. Jayasuriya, Drug transport mechanisms and in vitro release kinetics of vancomycin encapsulated chitosan-alginate polyelectrolyte microparticles as a controlled drug delivery system, Eur. J. Pharmaceut. Sci. 114 (2018) 199–209.
[168] C. Tan, J. Xie, X. Zhang, J. Cai, S. Xia, Polysaccharide-based nanoparticles by chitosan and gum Arabic polyelectrolyte complexation as carriers for curcumin, Food Hydrocolloids 57 (2016) 236–245.
[169] M. Song, L. Li, Y. Zhang, K. Chen, H. Wang, R. Gong, Carboxymethyl-β-cyclodextrin grafted chitosan nanoparticles as oral delivery Carrier of protein drugs, React. Funct. Polym. 117 (2017) 10–15.