Potentials of Hydrogels in Cancer Therapy

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Abstract: Hydrogels have emerged as an unique and potential transporters for the target drug delivery systems in cancer therapy. Cancer is a terrible disease extracting toll on human health across the world probably due to several drawbacks of the conventional cancer treatments. However, hydrogels facilitate the modern and improved prospects for the treatment of cancer with minimum cytotoxic effects to the healthier cells or tissues. Besides, the easy tailoring associated with the structural features and high biocompatibility of hydrogels have attracted enough attention and offer them as a strong tool for the development of nanomedicine. This review is devoted to update the current studies related to hydrogels and their applications as vectors for cancer chemotherapy.

1. INTRODUCTION

Discovery of drugs is considered as a landmark in the field of medical sciences [1, 2]. However, finding a bullet is not the end of the task, as a gun is required to transport this bullet to the target sites. Drug delivery is a domain that not only facilitates the delivery of drugs to the targeted tissues but also supports increased therapeutic actions by sustained release, regulation of drug delivery rate etc. [3, 4]. The functioning of drug delivery technologies are regulated by several factors *viz.* efficacy, safety and patient compliance [5]. Drug delivery equipment alters drug release mechanism, enhances product half-life, shelf life, absorption, distribution, elimination and reduces side-effects [6-10]. Hence, it is worth to say that drug delivery technology offers an economic and safe healthcare system [11].

In recent years, hydrogels have appeared as the potential drug delivery vehicles with their unique structure and swelling properties. Hydrogels can hold high amount of water and maintain their 3D structures [12-14]. Several features such as biodegradable [15, 16], biocompatible [17], enhanced water solubility, high payload capacity, pH dependent and controlled drug release [18], bioadhesive or targetable and self-regulated release of hydrogels advocate their strong candidature as controlled drug release systems [19]. Besides, soft and tissue like structural properties, release of entrapped molecules (plasmid-DNA (pDNA)/ short interference RNA (siRNA)/drug) in regulated fashion supported the potency of hydrogels in the area of biomedical science [20, 21].

In 1960, Wichterle and Lim for the first time developed hydrogels for biological purposes [22, 23]. Drug-polymer covalent conjugation came into highlight in 1975 [24]. According to SciFinder®, more than $1/5^{th}$ of published references (in between years 1950 to 2011) on hydrogel are belongs to drug delivery studies [23]. A literature survey indicated that hydrogels may have been designed and fabricated to achieve the need of pharmaceutical drug delivery vehicle with the aim to improve the system of medicine [25]. Hydrogels could be chemical or physical gels depending upon the nature of crosslinks present in the structure. In the former, crosslinking adheres molecular scaffold chains *via* covalent bonds resulting in irreversible hydrogels, however in the latter non-covalent bonds *viz.* electrostatic interactions, hydrogen bonding etc. cohere the unit chains. In physical gels, faster transition of sol to gel form without the formation of new covalent bonds supports their importance in medical applications [26]. Hydrogels can be employed in a number of ways for the treatment of cancer as shown in (Fig. **1**) [27].

Chemotherapy, a term put forth by Paul Ehrlich [28] is promising and better approach for the treatment of cancer over conventional cancer therapies like radiation and surgery. During the last few decades numerous anticancer drugs have been developed with poor understandings of their mode of actions [29]. However, conventional chemotherapy lacks tumor specificity as it destroys normal cells by cytotoxic effect of the drugs along with malignant cells, which leads to systemic toxicities and unwanted side-effects such as hair loss and adverse effects on bone marrow, kidney, liver and gastrointestinal epithelial cells [29, 30] even patient com-

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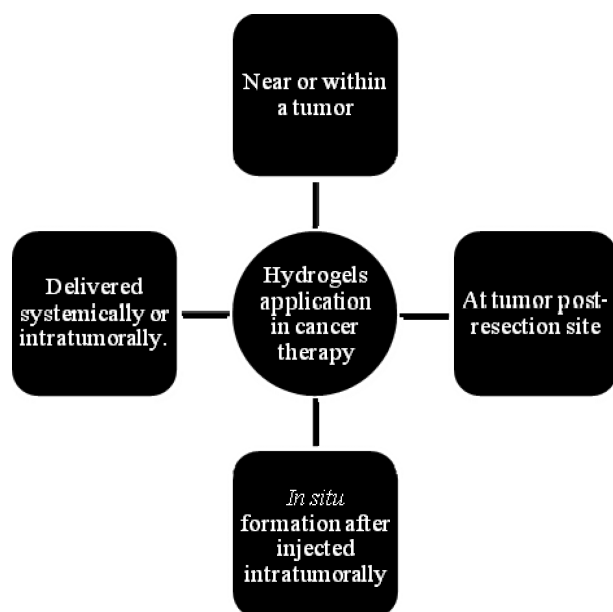
Fig. (1). Various routes employed for hydrogels in cancer therapy [27].

promises with their immune system [31]. Multi-drug resistant phenotypes associated with the anticancer drugs, is an another problem in cancer chemotherapy [32]. Moreover, chemotherapy is somewhat limited to younger patients as older patients are more affected in terms of independency, comorbidity and quality of life levels [33]. Hormonal therapy and immunotherapy are currently limited to certain types of cancer however, the former also increases the probability of cardiovascular disease (CVD) including tachycardia, urticaria, coronary artery disease (CAD) and diabetes [34-37]. There has been a demand of technologically enriched protocols for the better use of available anticancer drugs cleverly and with reduced wastage and side-effects. The controlled drug delivery approaches appeared as a new avenue for improved drug availability at target site by sustained release [38] and less frequent dosing [39] with poor chances of drug plasma concentration fluctuation [40]. The hydrophobic nature of most of the anticancer chemotherapeutics hampers their delivery in aqueous environment however, the problem is tackled by drug delivery approaches [41]. The collection of informations time to time in this area is a crucial step for further development of cancer chemotherapy. This review highlights the updates on hydrogels and their potentials in cancer chemotherapy. The phenomena like enhanced permeability and retention and modes of drug release have also been taken in to description. Besides, hydrogel based delivery of monodrug, two drugs at same time and chemoimmunotherapeutic agents, thermosensitive, light sensitive, magnetothermal, ion sensitive, pH sensitive hydrogels, lipogels, antigen sensitive hydrogels, receptor mediated drug delivery, etc. have been described.

2. CLASSIFICATION OF HYDROGELS

Due to unique swelling properties in response to different stimulus and biocompatibility, hydrogels are competent and fashionable candidates for the drug delivery system. Simoes *et al.* classified hydrogels in various classes as follows [42].

- Origin (natural, synthetic)

- Ionic charge (neutral, anionic, cationic, ampholytic)

- Water content or degree of swelling (low swelling, medium swelling, high swelling, superabsorbent)

- Structure porosity (nonporous, microporous, macroporous, superporous)

- Network morphology (amorphous, semicrystalline, hydrogen bonded structures, super molecular structures, hydro colloidal aggregates)

- Crosslinking method (chemical/covalent, physical/non-covalent)

- Component of polymers (homopolymer, multipolymer, interpenetrating)

- Function (biodegradable or non-biodegradable, stimuli responsive, superabsorbent)

The various natural and synthetic polymers that are used as building blocks for hydrogel nanoparticles (HNPs) in hydrogel system include; natural polymers *viz.* gelatin, and chitosan and synthetic polymers *viz.* poly(vinyl alcohol) (PVA) and poly(ethylene oxide) (PEO) [43]. Self-assembled peptides fall under natural and synthetic categories. In natural category, peptides are arranged into basic conformation such as β-sheets and turns, α-helices and coiled coils while synthetic categories (peptide-amphiphile and aromatic peptide) possess amino acids functionalized with other molecules [44].

Electrosensitive hydrogels are another class of hydrogels possessing potential as drug carriers which can undergo shape alteration when subjected to applied electric field [45]. Hydrogel swelling, shrinking and bending depends upon several factors including surface of the hydrogel whether it is in contact with the electrode or suspended in the water (acetone-water mixture) without contacting the electrode. An infinitesimal change in electric potential across partially hydrolysed poly(acrylamide) (PAAm) hydrogel which are in contact with both the anode and cathode undergoes volume collapse, leading to loss of water at the anode side due to H^+ migration towards cathode. Concurrently, negatively charged acrylic acid (AAc) groups moved towards the anode surface due to electrostatic attraction creating a uniaxial stress along the gel axis. These two events together make hydrogel to shrink from anode side [46, 47]. Hence, control of 'on-off' drug release is achieved by varying the intensity of electric stimulation. Ramanathan *et al.*, investigated the effect of electric current on neutral, anionic and cationic drug release from hydrated chitosan hydrogel (CH) [48]. Unfortunately, no study has been reported on anticancer drug release from these hydrogels under the influence of electric field so further experiments become necessary to develop electrosensitive drug delivery modules that work under physiological conditions.

3. ENHANCED PERMEABILITY AND RETENTION (EPR) EFFECT

Now a days enhanced permeability and retention (EPR) concept is considered as unique standard for the design of new anticancer drugs [49]. Bamrungsap *et al.* reported that
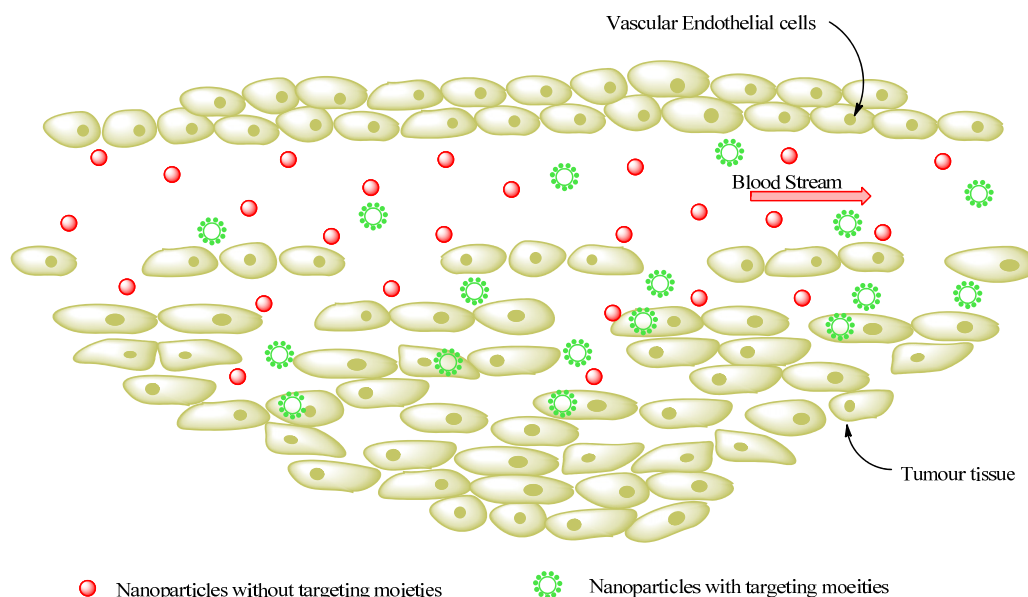
Fig. (2). The targeting strategies for cancer therapy by passive (🔴) and active (🟢) targeting of cells. Modified with permission from ref. [57].

tumor vessels are generally disorganised in membrane structure and dilated with high number of pores that leads to the formation of large gap junction between endothelial cells [50]. It is also noted that the microvasculature is not uniform in all tumors such as the pore size in brain tumor is ~7 nm while it is ~50 to 60 nm in pancreatic tumor [51]. This compromised lymphatic drainage results in movement of drugs by passive or active mechanism more easily, that is leaky vascularization refers to EPR effect. The passive targeting process involves drug release in the extracellular matrix followed by its migration to the tissues by diffusion. However, complex structures of few drugs do not allow diffusion so efficiently and also some tumors do not have an EPR effect, hence active targeting process is more appealing. Ligands (aptamers [52], Antibodies [53], folate ligands [54] and peptides [55]) on nanocarrier surface increases their affinity to bind with cancer cell receptors fall under active drug targeting with enhanced drug internalization by receptor mediated endocytosis [50, 56]. With incorporation of above cell specific ligands, nanocarriers are capable to deliver cancer therapeutics to cancer cells *via* active and passive process through EPR effect (Fig. **2**) [57].

4. MODES OF DRUG RELEASE

The ratio of hydrophobic and hydrophilic groups in hydrogel is the crucial and rate limiting step for the process of controlled release of drugs. Based on mechanisms, drug can be released by various mechanisms [58]. In diffusion-controlled systems, drug is released along with concentration gradient in accordance to Fick's law [42, 59]. The rate of drug release depends on the membrane permeability and device configuration. If drug activity inside the membrane is constant and infinite sink conditions are maintained, then drug release is time independent and affords zero-order kinetics. The hydrodynamic and obstruction based theories may be useful to analyse drug diffusion [60].

A swelling-controlled system refers to drug diffusion faster than hydrogel swelling [42, 59] which results from glassy state to rubbery state transition of polymer. Entrapped drug molecules are immobile in glassy state while they diffuse rapidly through the rubbery state of swollen polymer. In this system, glass-rubber polymer transition temperature is lower than the drug delivery matrix surrounding fluid. Hydrogel matrix is divided into a glassy and swollen region when released fluid molecules contact the external layer of the hydrogel. Hydroxypropyl methylcellulose (HPMC) (Fig. **3**) hydrogel tablet is one of the examples which follow this mode of drug release [61, 62]. Drug release depends upon polymer chain cleavage by hydrolytic/enzymatic degradation reactions and reversible/ irreversible reactions come under *chemically-controlled system* [63, 64]. In modulated release systems, the drug release is governed by external stimuli such as temperature, ionic strength, pH etc. [58].

5. APPLICATIONS OF HYDROGELS IN CANCER CHEMOTHERAPY

The normal body of human beings maintains a balance between cell division (proliferation) and cell death (apoptosis) however, irregularties associated with these processes invite over-proliferate of cells without any control and ultimately leads to tumor formation. [65-68]. A number of fac-
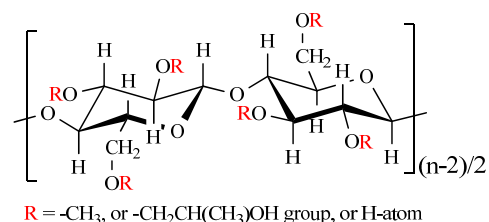

R = -CH$_3$, or -CH$_2$CH(CH$_3$)OH group, or H-atom
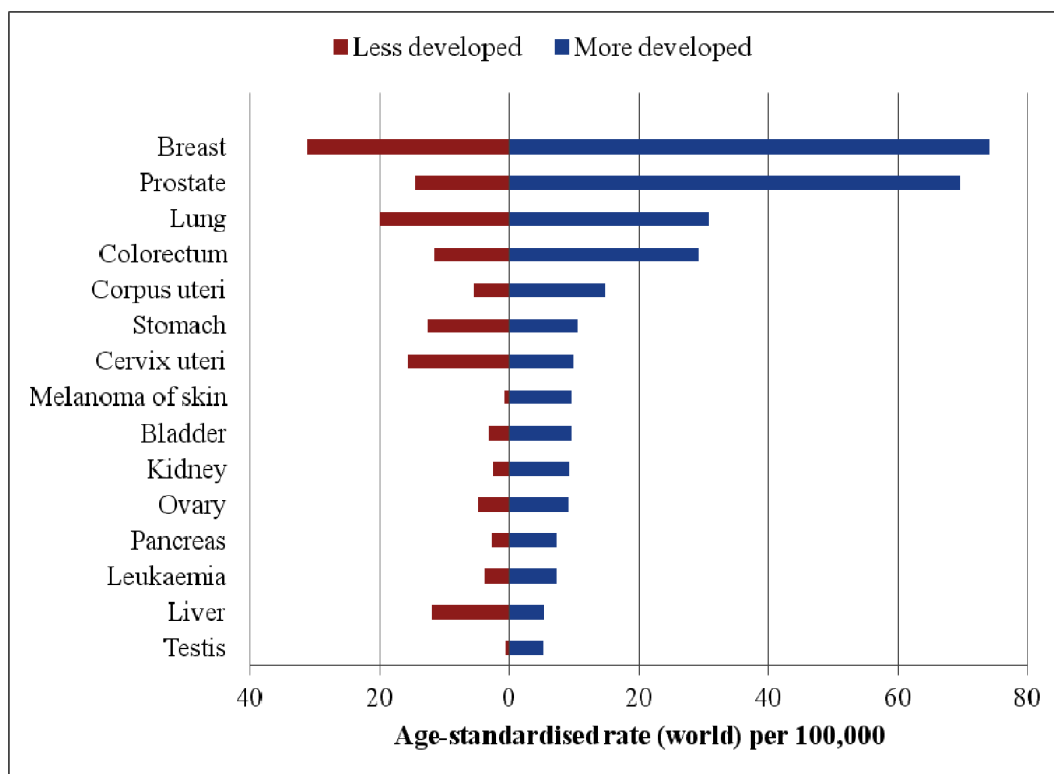
Fig. (3). The chemical structure of HPMC.

Fig. (4). Statistics of cases of various types of cancers in developed and less developed regions [75].

tors influence the development of cancer which includes both *endogenous* (age, hormone production, genes inheritance, etc.) and *exogenous* factors (radiations, mutagens etc.). Older people are more prone to develop cancer than younger ones excluding some malignant diseases in children [69]. Endometrium, breast, prostate, ovary, testes, thyroid and bone cancer are known to be linked to hormones [69, 70]. Retinoblastoma causing gene (RB1) has been identified to pass from generation to generation causing retinoblastoma in subsequent generations [71]. Ultraviolet light and all ionizing radiations are connected to skin cancers [72]. People frequently exposed to metals (especially As and Pb) and chemicals like pesticides (DichloroDiphenylTrichloroethane (DDT) and Ethylene dibromide (EDB)) in metal-smelting industry and food articles respectively, are prone to develop certain cancers [73, 74]. According to *International Agency for Research on Cancer (WHO),* people in developed countries suffer more with cancerous disease than people in developing countries (Fig. **4**) [75].

It is also estimated that in United States of America, slightly less than a 1 in 2 men and little more than 1 in 3 women will acquire cancer during their lifetime [76] which could be due to their lifestyle, irregularities in daily schedule, more machinery convenience and lack of physical exercises.

The conventional cancer treatment *viz.* surgery, radiotherapy and chemotherapy are not perfect treatment due to several drawbacks. Nearly 10 to 50 % of the breast cancer survivors by surgery and radiotherapy develop lymphedema of the arms [77] and infertility [78]. In certain leukaemia, children may acquire cognitive deficits upon cranial radiotherapy [79]. Most importantly, there is a possibility of reap-

pearance of tumor after surgically removed tumors [80, 81]. A number of commercially available hydrogels include yearly administered histrelin acetate hydrogel, used in the treatment of prostate cancer under the trade name of 'Vantas' [82]. It is also noteworthy that non-spherical (rod-like or disc-shaped) nanoparticles have better penetration and accumulation ability due to shortest dimension of the particle than size-matched spheres [51].

An ideal drug carrier hydrogel should releases drug in response of some stimulus. Stimulus are categorised into three classes *viz.* physical, chemical and biological stimuli. Hydrogels responding to these stimuli are called smart hydrogels with sensor function, processor functions and actuator functions [12].

5.1. Physical Stimulus

Hydrogel shows swelling and deswelling characteristics under the influence of physical stimulus. The physical stimuli could be temperature, light, alternate magnetic field (AMF), electric field and on this basis hydrogels have been divided into smart categories.

5.1.1. Thermosensitive Hydrogels

Temperature sensitive hydrogels are the most explored one for chemotherapeutic drug delivery purposes. Hydrophobic groups such as methyl, ethyl *etc.* are common substituents found in thermosensitive hydrogels. These temperature-sensitive polymers especially poly(N-isopropylacrylamide) (PNIPAAm) and poly(diethylacrylamide) (PDEAAm) exhibit a lower critical solution temperature (LCST) close to the body temperature, below which the polymer is soluble in water [83] and *vice-versa.* Sol-gel phase transition of these

polymers is influenced by the balance between hydrophilic and hydrophobic interactions [47]. The entropy of the water is the main driving force in sol-gel transition which depends on temperature. Water is less ordered with higher entropy when the polymer is in gel form. This is also known as the "hydrophobic effect" [84, 85].

Thermosensitive hydrogels are categorised into three classes [47] *i.e.* negative thermosensitive, positive thermosensitive and thermoreversible hydrogels. Negative thermosensitive hydrogels show lower LCST that is the critical temperature below which polymer and solvent are completely miscible, (Fig. **5A**) [86]. These hydrogel swell at temperature below to LCST. Hydrogen bonding between hydrogel monomers and water molecules is the driving factor for swelling, while hydrophobic interactions between hydrogel monomers become high as temperature rises above LCST which leads to shrinking of hydrogel [47, 86]. Drug is released due to hydrogel swelling (on state) making it more permeable.
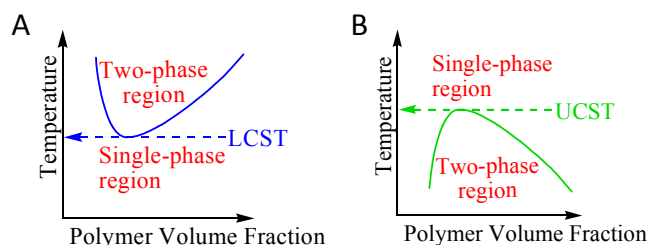

Fig. (5). Temperature *vs.* polymer volume fraction: Schematic illustration of phase diagram for polymer solution showing phase change in response to (**A**) LCST and (**B**) UCST. Modified with permission from ref. [86].

The PNIPAAm (Fig. **6A**) is a well-known negative thermosensitive polymer (LCST 32 °C) [58]. In squeezing mechanism, drug is released at high temperature due to shrinking of gel. Hydrogel of poly-organophosphazene polymers possessing α-amino-ω-methoxy-poly-(ethylene glycol) (AMPEG) and hydrophobic L-isoleucine ethyl ester side groups have been studied for its drug release profile with the loaded drug doxorubicin (DOX) (Fig. **6B**). Sustained drug release was observed over a period of time without disturbing its gel strength [87].

The poly(ethylene glycol) (PEG) is highly used polymer in cancer chemotherapy because it enhances hydrophobic drug solubility in aqueous solution due to PEGylation [88]. Lin *et al.,* prepared injectable and thermosensitive PLGA-g-PEG/HA hydrogel composites comprised of poly-(lactic acid-co-glycolic acid)-g-poly-(ethylene glycol) (PLGA-g-

PEG) and hydroxyapatite (HA). PLGA-g-PEG/HA hydrogel (30%:10%::PLGA-g-PEG:HA) adopts sol state at 4 °C and turns to gel at 37 °C (Fig. **6C**). The storage modulus (G') of the hydrogel was found to increase with increase in HA concentration across temperature range examined (15-50 °C). This hydrogel release dye *in vitro* in sustained manner for at least two weeks [89].

Positively thermosensitive hydrogels swell at temperature higher than upper critical solution temperature (UCST) *i.e.* the hydrogel shrinks when cooled below UCST (Fig. **5B**). Above this critical temperature polymer and solvent are completely miscible [86].

Thermoreversible hydrogels show cyclic sol-gel phase transitions. These hydrogels are used with the liposomes in order to deliver 5-fluorouracil (5-FU) due to its hydrophilic nature and to increase its half-life with sustained release [90]. Besides, this is used to incorporate vector-containing gene responsible for enhanced antitumor immune response and to minimize vector dissemination related issues [91].

5.1.1.1. Role of Thermosensitive Hydrogels in Chemotherapy

Thermosensitive hydrogels are widely used in the delivery of the chemotherapeutic agents. Collagen-poly(2-hydroxyethyl methacrylate) (collagen-pHEMA) hydrogels loaded with 5-FU, bleomycin A2 (BLM), and mitomycin C (MMC) were reported to follow zero-order kinetics for drug release and time independence at pH and temperature 7.4 and 37 °C, respectively and hence can be employed as control release drug carriers [92]. Cheng *et al.* prepared 5-FU loaded GA-CH/5-FU hydrogel composed of hepatoma cell-specific binding molecule glycyrrhetinic acid (GA) and CH. Hydrogel, GA-CH/5-FU reveales dose and time-dependent anticancer effects with the release of 5-FU in sustained-release manner with three distinct phases such as quick, steady, and slow. It is noticed that GA-CH/5-FU significantly increases survival time by inhibiting cell proliferation in orthotropic liver cancer mouse model (Fig. **7A**) [93].

Kushwaha *et al.* prepared chitosan/β-Glycerophosphate/HP-β-Cyclodextrin (C/β-GP/HP-β-CD), a temperature sensitive hydrogel with entrapped camptothecin (CPT) drug molecules. Interestingly, CPT showed good release *in vitro* profile with significant tumor inhibition against MFC7 tumor cells [94]. The CPT encapsulated hydrophobically modified glycol chitosan (CPT-HGC) nanoparticles were reported by Min *et al.* in 2008 [95]. The HGC nanoparticles (Fig. **7B**) showed the drug loading efficiency above 80% and up to 45% initial release in 9 hours with sustained release for one
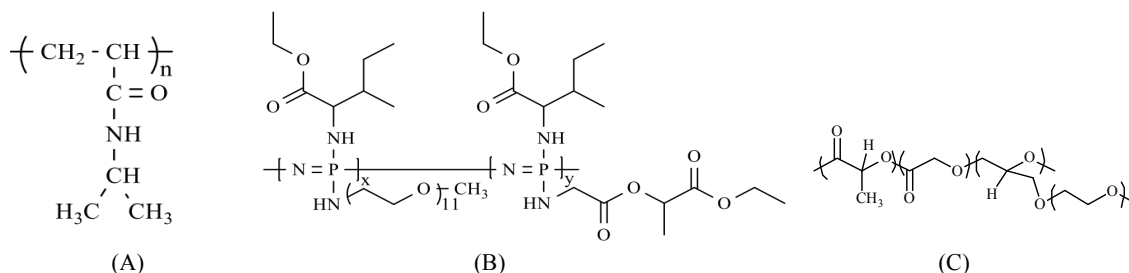

Fig. (6). (**A**) PNIPAAm structure; (**B**) Poly-organophosphazene Polymer Structure; (**C**) PLGA-g-PEG Structure.
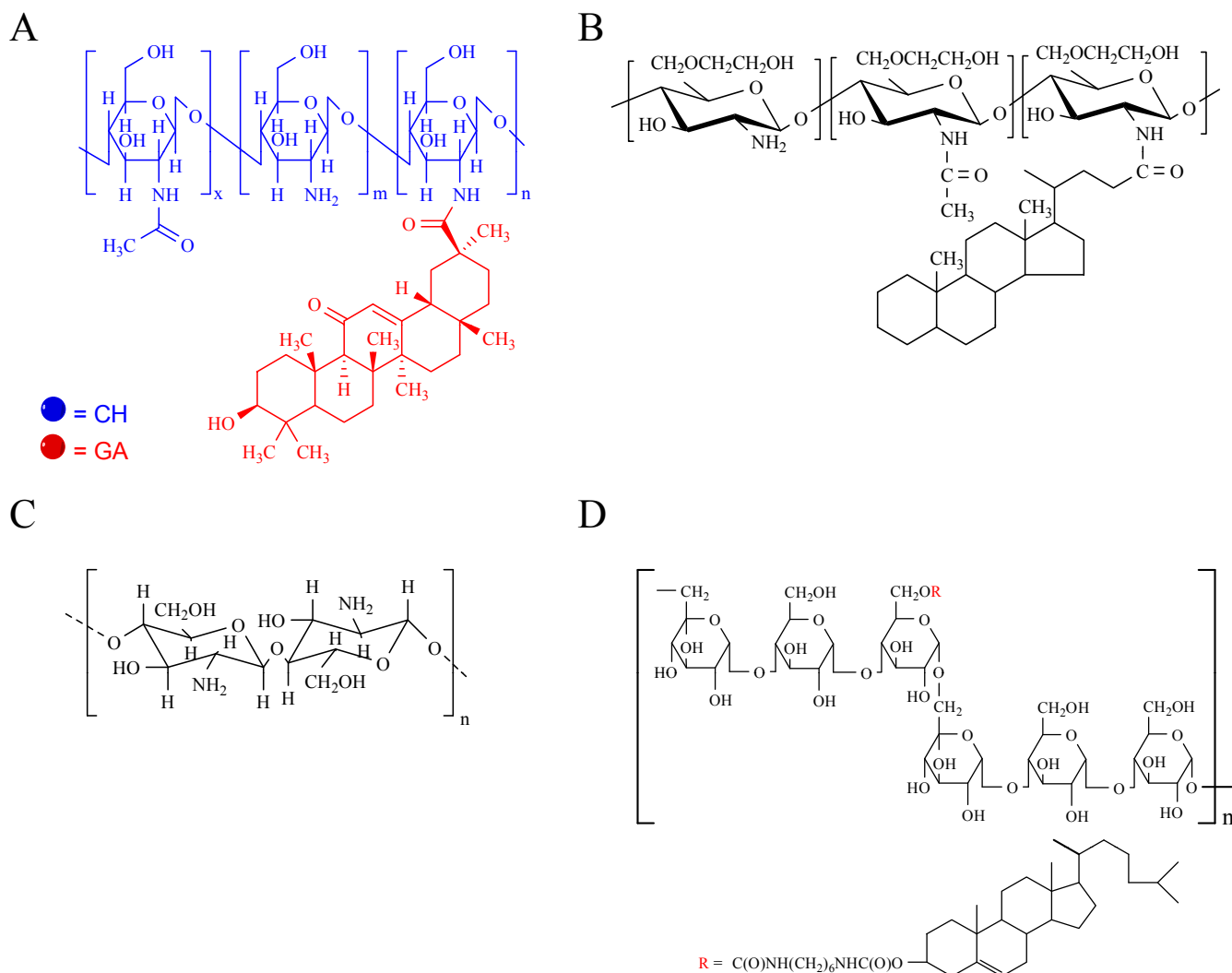
Fig. (7). (**A**) GA-CH Structure; (**B**) HGC Structure; (**C**) CH Structure; (**D**) CHP Structure.

week and this kind of phenomenon is called a biphasic release [96]. CPT-HGC showed significant antitumor effect with high specificity towards MDA-MB231 human breast cancer xenograft in nude mice.

Other examples include paclitaxel (PTX) incorporated CH/ β-GP hydrogel where CH (Fig. **7C**) is stabilized by β-Glycero phosphate (β-GP) and observed *in vitro* PTX sustained delivery over one month and *in vivo* antitumor activity in Balb/c mice against EMT-6 cells [97]. Azide-chitosanlactose (Az-CH-LA), a photocrosslinkable hydrogel loaded with PTX showed tumor inhibition in Lewis lung cancer (3LL) cells bearing male C57 BL/6 mice [98]. It was also observed that the pressure sensitivity is a common characteristic of temperature sensitive gels. The pressure sensitivity of the temperature sensitive gels was attributed to an increase in their LCST values with pressure. The pressure sensitive hydrogels undergo expansion and compression at high and low pressure, respectively [99]. The PNIPAAm hydrogel showed increase in degree of swelling as the temperature is closed to its LCST under hydrostatic pressure [100].

5.1.1.2. Role of Thermosensitive Hydrogels in Gene Therapy

Gene therapy is the treatment of disease through the use of exogenous DNA segment, genes, portion of gene, oligonucleotides or RNA introduced inside the cells to alter the phenotype of a diseased cell. This technology is emerged to improve the efficacy and minimize toxicity of conventional chemotherapy. The temperature responsive hydro-gels such as CH possessing nominal inflammatory response [101] have been employed for siRNA delivery [102].

Transglutaminase-2 (TG-2) is overexpressed in the cancer cells which mediates drug resistance through the activation of survival pathways and evasion from apoptosis but also regulating extracellular matrix (ECM) formation [101, 103]. TG-2 was targeted using siRNA so that cells become sensitive to the anti-cancer drugs such as docetaxel (DTX). Alexa555 siRNA/CH hydrogel system was checked for *in vivo* delivery by a single intratumoral injection into A375SM-bearing mice. In addition, CH loaded with DTX was also prepared. *In vivo* efficacy of siRNA with its sustained release was determined in mice by using these preparations. It was noticed that the antitumor activity increased

significantly when above mentioned preparations were used together [102].

Apart from being thermosensitive, hydrophobic [104] chitosan nanoparticle loaded with naked supercoiled p53 DNA was found significantly effective as delivery vehicle for p53 to HeLa (Human negroid cervix epitheloid carcinoma) and A549 (non-small lung carcinoma cell line) cell and restore p53 mediated apoptosis. Moreover, the cell viability was not disturbed by these nanoparticles [105].

Megeed *et al.* found that plasmid molecular weight/size influences its release from the silk elastin-like protein (SELP-47K) Polymer. The smallest and lightest weight plasmid, pUC18 (2.6 kbp) was released fastest, followed by pRL-CMV (4.08 kbp), pCFB-EGSH-Luc (8.5 kbp), and the largest plasmid pFB-ERV (11 kbp). It was found that DNA purified from the hydrogel did not lose its bioactivity significantly even after the incubation in phosphate buffer solution (PBS) at 37 °C for 28 days. The delivery (*in vivo*) of *Renilla luciferase* plasmid from SELP-47K in athymic nude mouse/MDA-MB-435 breast cancer showed enhanced gene expression upto 21 days in tumor than plasmid alone [106].

5.1.1.3. Role of Thermosensitive Hydrogels in Immunotherapy

Immunotherapy is an emerging area in cancer therapy through which body defence mechanism can be evoked above a threshold level. The various growth stimulating factors such as granulocyte-macrophage colony-stimulating factor (GMCSF) and cytokines possess the potentials to stimulate the body immune response. GMCSF loaded CH along with various chemotherapeutic drugs *viz.* doxorubicin (DOX), cisplatin (CDDP), and cyclophosphamide (CTX) were used as chemo-immunotherapeutic agents (CH-a cancer drug + GMCSF) to observe its effect on TC-1 cervical tumor cells expressing tumor specific antigen HPV-16E7 in C57BL/6 mice. The combination of CH, a cancer drug and GMCSF was noticed to reduce TC-1 tumor significantly as compared to those mice treated with CH alone or GMCSF (CH-GMCSF). The combination therapy of CH-CTX and GMCSF was also associated with the induction of E7-specific CD8$^+$ T cell immune response [107].

Interleukin 12 (IL-12) has anti-angiogenic effects in melanoma cells [108]. Shimizu and co-workers employed interleukin-12 (IL-12) into cholesterol bearing pullulan (CHP) nanogel (Fig. **7D**). The recombinant murine IL-12 (rmIL-12) was incorporated into CHP based hydrogel, incubated at 25, 37, and 60 °C for various time durations (10 min to 36 hrs). CHP/rmIL-12 complex was found to be highly efficient when the combination of CHP and rmIL-12 was incubated at 37 °C for long periods (0.5-2 hrs). The time course of IL-12 level was observed to be dependent upon route of administration. The serum level of IL-12 (CHP/rmIL-12 nanogel injected intraperitoneally) reached peak at 1 h followed by a sharp decrease as compared to subcutaneous and intravenously administration. CHP/rmIL-12 nanogel was further injected subcutaneously into mice which gave prominent retardation of subcutaneous fibrosarcoma than CHP/BSA and rmIL-12. There was no activity impairment and no significant toxicity, after being released from CHP [109].

The combination of CH-DOX hydrogel with vaccinia virus vaccine expressed Sig/E7/LAMP-1 (Vac-Sig/E7/LAMP-1) and was projected as the chemo-immunotherapeutic agent [110]. The intratumoral injection of CH-DOX hydrogel showed synergistic effects on tumor retardation with vaccinia virus vaccine than free DOX. The vaccination during 3 days before tumor treatment with CH-DOX, decreased tumor greatly, while vaccination at 0 or 3rd day after treatment with CH-DOX failed. The combination of CH-DOX and Vac-Sig/E7/LAMP-1 led to the highest tumor suppression than CH plus Vac-Sig/E7/LAMP-1 or CH-DOX plus Vac-W.T. (Wild type vaccinia virus). The CH-DOX and Vac-Sig/E7/LAMP-1 together as chemo-immunotherapeutic agent enhanced the number of E7-specific IFN-γ-secreting CD8$^+$ T cells in comparison to that of CH- or CH-DOX plus wild type vaccine controls, PBS alone or CH(empty) + Vac-Sig/E7/LAMP-1 vaccine. This chemo-immunotherapy had potent tumor growth suppression effects at least up to 60 days after tumor challenge.

N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium methyl sulfate (DOTAP), deguelin (D), monomethoxy poly(ethylene glycol)-poly(3-caprolactone) (MPEG-PCL) or (MP) (Fig. **8A**), and pluronic F127 [F-127] (Fig. **8B**) (thermosensitive hydrogel, sol at 4 °C and gel at 37 °C) have been used to prepare D/DMP-F-127 hydrogel. The D/DMP-F-127 hydrogel extended the residence time of hydrophobic drug (deguelin) to a significant extent and increased drug concentration inside of bladder. The cold D/DMP-F-127 (below 20 °C) sol was administered intravesically into urine bladder which turned into gel at 37 °C and acted as a drug depot and resisted elimination after a single urination [111]

5.1.1.4. Thermosensitive Hydrogels in Radiation Therapy

Temperature sensitive hydrogels are used in radiation therapy for the treatment of cancer. Radio-nucleotide β-emitters (^{188}Re) damage biomacromolecule, which leads to cancer cell apoptosis [112]. The polymer solution of a weak base β-GP and chitosan aqueous solution remains in solution state at room temperature and neutral pH and turned into homogenous gel on increase in temperature [113]. Huang and co-workers combined CH and β-GP to achieve CH/GP hydrogel for the controlled delivery of ^{188}Re-Tin colloids, a new internal radiation therapy method [114].

5.1.2. Light Sensitive Hydrogel

The light sensitive hydrogels are more advantageous than others as the property of the light stimulus to be imposed instantly and its delivery in specific amount with high accuracy [47]. Among the numerous chromophores applied in light-responsive hydrogels, o-nitrobenzyl is of particular importance since it can undergo a photolysis reaction under either UV light or near-infrared light, disrupting the hydrophilic-hydrophobic balance of the polymer containing o-nitrobenzyl and thus the integrity of assemblies [115, 116]. A light-sensitive chromophore (*e.g.* trisodium salt of copper chlorophyllin, Fig. **8C**) was introduced into PNIPAAm hydrogel in order to prepare visible light-sensitive hydrogels [99, 117].

An increase in temperature is directly proportional to the light intensity and the concentration of the chromophore. Hydrogels possessing chromophore absorb light during ex-
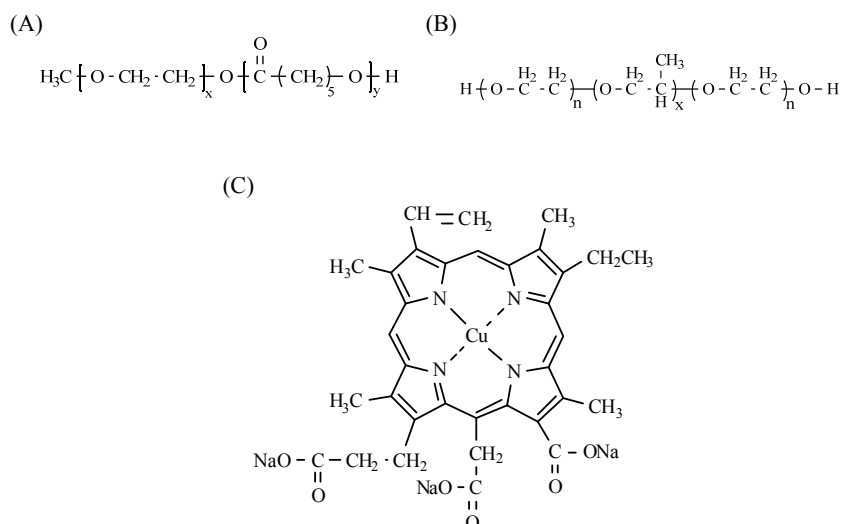
(A)

(B)

(C)

Fig (8). (**A**) MPEG-PCL structure; (**B**) Pluronic F-127 Structure; (**C**) Trisodium salt of copper chlorophyllin Structure.
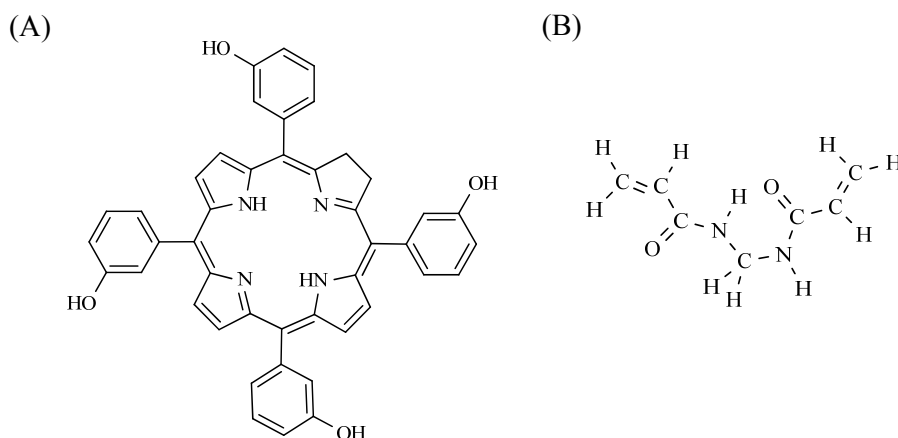
(A)

(B)

Fig. (9). (**A**) The chemical structure of mTHPC; (**B**) The chemical structure of N,N-methylenebis(acrylamide) (MBA).

posure and dissipated locally as heat. Thus, increase in hydrogel temperature leads to the swelling behaviour alteration of light sensitive hydrogel. The restructuring of polymer chains upon temperature change and leaching of chromophore during swelling/deswelling cycle limits the use of these hydrogels [47].

A new therapy namely Photodynamic therapy (PDT) has emerged as an important therapy for the treatment of certain types of cancer [118]. In PDT, a specially designed photosensitizer molecule which is localized in the cancer tissue upon optically activation, transfer its energy to surrounding molecular oxygen which in turn changes into highly reactive singlet oxygen species ($^1O_2$). The formation of such highly reactive species kills tumor cells either by necrotic or apoptotic pathway or microvascular injuries [119]. A combination of poly(ethylene glycol) double acrylates (PEGDA) hydrogel and TiO_2 nanorods with diameter of ~5 nm and length of ~25 nm were found to produced high concentration of singlet oxygen ($^1O_2$) under NIR irradiation, which induced apoptosis of tumor cell. Hydrogel not only retain the TiO_2 nanorods around tumor cells but also prevent migration of TiO_2 nanorods from tumor to normal tissue [120].

Meta-tetra(hydroxyphenyl)chlorine (mTHPC) (Fig. **9A**) are one of the "second-generation" photosensitizers that has been approved for neck and head cancer therapy in European union, due to its high phototoxicity at very low concentration or at low light levels [118, 119]. Acrylamide monomers (AAm), N,N-methylenebis(acrylamide) (MBA) (Fig. **9B**) crosslinker and mTHPC with prior treatment of methanol were emulsified into a non-polar solution to encapsulate hydrophobic drug molecule into ultrafine hydrophilic drug nanoparticle. The nanoparticles loaded with mTHPC were found effective against rat C6 glioma cells at 650 nm light even at a low concentration [121].

Konan *et al.*, incorporated meso-tetra(4-hydroxylphenyl)porphyrin (mTHPP) into sub-200-nm silica or poly-(lactic-co-glycolic acid) (PLGA) nanoparticles. However, the issues related to size, surface characteristics and its application in biological environment remains unspoilt [122].

5.1.3. Magnetothermal Hydrogels

Tumors consist of immature vascular system as compared to healthy cells and are more susceptible to heat [123-125]. When certain organs or tissues are subjected to tem-
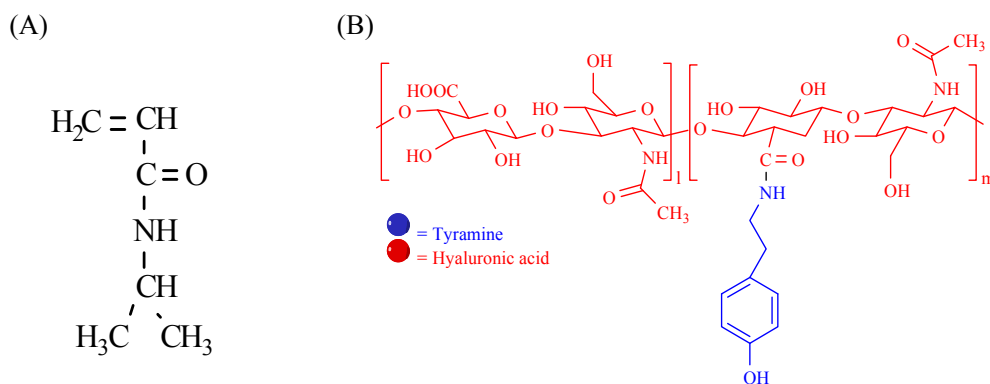
Fig. (10). (**A**) The chemical structure of NIPAAm; (**B**) HA-Tyr Conjugates Structure.

perature between 41 °C and 46 °C preferentially for cancer therapy, the process is called as hyperthermia [126]. Hyperthermia is the application of heat to kill cancer cells. It should be noted that heat of the affected cells cannot be removed efficiently and normal cells remain less affected [127]. In early clinical usage of hyperthermia to treat cancers, heat is generally applied to whole body by using heated blankets or by immersing whole body in water baths. However, whole body hyperthermia leads to serious side effects. In 1957 Gilchrist *et al.* [128] put forward the magnetic fluid hyperthermia treatment (MFH), in which AMF is applied exterior to the body surface to specifically heat the magnetic nanoparticles [129].

The high temperature range above 50 °C, which yield widespread necrosis, coagulation or carbonization (depending on temperature) is called thermoablation [126, 130]. Meenach *et al.*, presented PEG based hydrogels and investigated their biocompatibility, heating capabilities, temperature-responsiveness swelling, mechanical strength and ability to deliver paclitaxel with ability to kill M059K glioblastoma cells *via* thermoablation. It was observed that thermoablation temperature of 63 °C efficiently kills M059 cells with no toxicity caused by magnetic field [130]. Brownian movement and Neel relaxation losses are the bases for the heating of superparamagnetic iron oxide nanoparticles which are loaded in hydrogel and create tunable nanocomposites that can be remotely controlled by external AMF. The magnetite (Fe_3O_4, iron ferrite) is one of the primary magnetic material investigated however, others magnetic materials include ferrimagnetic (Magnetite Fe_3O_4), ferromagnetic (Fe, Co, Ni), antiferromagnetic (MnO, FeO), paramagnetic (Na, Al) and diamagnetic (Cu, He) [131]. Some other include Hematite (Fe_2O_3) [132], Copper nickel (CuNi) [133], Iron palladium (FePd) ($L1_0$ crystals) [134] and Cobalt platinum (CoPt) ($L1_0$ crystals) [134]. It is recommended to evaluate the cytotoxicity of these magnetic materials before they are used in making magneto-thermally responsive hydrogel. Curie temperature is one of the unique properties of magnetic material so careful material selection is required to get on/off thermoresponsive delivery system [127]. When AMF is applied, magnetic material undergoes heating up until their Curie temperature is achieved. When the Curie temperature is reached, the heating stopped due to saturation of magnetization of particles drops to zero [127, 135]. Hence, selection of particle composition and size are crucial to fix curie tempera-

ture so that hyperthermia can be applied carefully without excessive temperatures. Satarkar *et al.*, has successfully demonstrated remote controlled heating and drug release by using negative temperature sensitive hydrogel nanocomposites based on NIPAAm (Fig. **10A**) and Fe_3O_4 superparamagnetic nanoparticles. Once AMF is applied, NIPAAm-Fe_3O_4 system temperature rises, leading to squeezing out of imbibed drug [136].

Ang *et al.*, also used PNIPAAm gel (LCST: 32 °C) with Fe_3O_4 system for dual purposes *viz.* hyperthermia and triggered drug release [137]. The concentration of magnetic particles and magnetic field strength influence the maximum temperature attained and gel turns to collapse at 34 °C. PNIPAAm-Fe_3O_4 system was found to be the best for 2.5 wt% Fe_3O_4 in PNIPAAm, which was heat7ed to 45 °C within 260 seconds under magnetic field of 1.7 kA/m. Also, the specific absorption rate (SAR) of Fe_3O_4 was found 1.83 times higher than iron (0.67) [138].

5.1.4. Drug Release Regulated by Concentration Gradient

Xu *et al.*, prepared interferon-α2a (IFN-α2a) incorporated hyaluronic acid-tyramine (HA-Tyr) (Fig. **10B**) hydrogel for liver cancer therapy where stiffness of IFN-α2a incorporated HA-Tyr hydrogels is influenced by hydrogen peroxide (H_2O_2) concentration. The release profiles of IFN-α2a from HA-Tyr hydrogels were studied by enzyme-linked immunosorbent assay (ELISA) where rapid release was observed due to the difference between protein's concentration of the interior of the hydrogel and its external environment. The cumulative releases of IFN-α2a from HA-Tyr-soft-IFN and HA-Tyr-stiff-IFN after 8h reached a plateau and were 78.5±1.4% and 46.0±1.7%, respectively. The pharmacokinetics studies (*in-vivo*) of IFN-α2a in plasma revealed prolonged and continuous release of IFN-α2a from HA-Tyr hydrogels than IFN-α2a alone. IFN-α2a doesn't affect the rheological properties of hydrogel. IFN-α2a incorporated HA-Tyr hydrogels significantly inhibited liver cancer cells proliferation and induced apoptosis through caspase-3/7 pathway *in vitro* than IFN-α2a solution (not effective). This hydrogel is also effective against angiogenesis of tumor tissue [139].

5.2. Drug Release Governed by Chemical Stimulus

In this type of hydrogel system, chemical stimulus like presence of various ions and acidic or basic surrounding me-
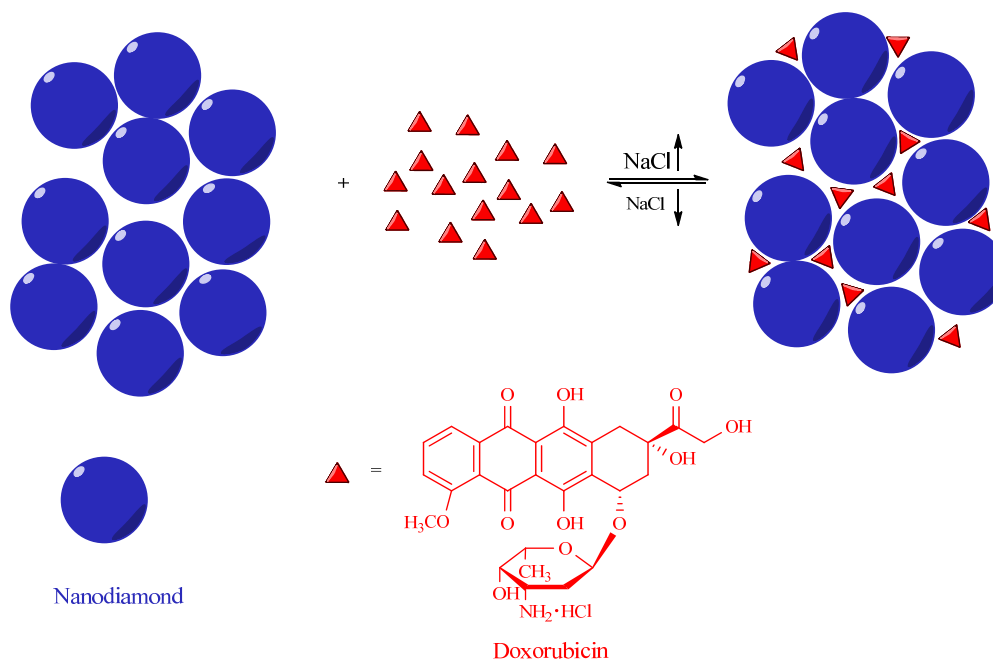
Fig. (11). DOX-NDH Complex. Modified with permission from ref. [142].

dium is responsible for the sol gel phase transition and drug release.

5.2.1. Ion Sensitive Hydrogels

In ion sensitive hydrogels, phase transition is influenced by the presence of monovalent (K^+, Na^+) and divalent cations (Ca^{2+}, Mg^{2+}) [140, 141]. Huang *et al.*, developed nanodiamond hydrogel (NDH) materials for the delivery of DOX-hydrochloride (DOX-HCl) towards human colorectal carcinoma cells. However, ND ions (ND-COO$^-$) are negatively charged and DOX ions (DOX-NH$_3^+$) are cationic, DOX ions are not easily adsorbed by the NDH due to high aqueous dispersibility of both the ions. The increased concentration of NaCl increases formation of DOX-NDH complex (Fig. **11**) as there is an increase in Cl$^-$ ions. These hydrogels exhibit reversible release of DOX by regulating Cl$^-$ ion concentration [142].

Feng *et al.*, found that elastic modulus of hydrogel can be tuned by ionic strength because ionic strength increases linearly with logarithm of its ionic strength [143]. Skouri *et al.*, observed that poly(acrylic acid) (PAA) gels shear modulus elevates with the addition of salt and this effect was attributed to the screening of the electrostatic repulsion by the same [144].

5.2.2. Hydrogels Sensitive to Acidic or Basic Environment

In tumor tissues, the extracellular pH (pHe) environment is known to be lower than the normal tissues, which encourage scientists to design drug carriers for the efficient use of drugs [145-147]. The extracellular pH ranges vary with different tumor tissues of various cancer types such as astrocytoma has pHe range 5.8 to 7.1 and uterine cancer has pHe range between 6.5 and 7.2 [145]. Depending upon surroundings, hydrogels are acid sensitive and release drug in either extracellular (slightly acidic pH in tumor tissue) or intracellular (in acidic endosomes or lysosomes) manner [148]. These hydrogels are of two types; anionic and cationic hy-

drogels. The anionic hydrogels are composed of anionic pendent groups (carboxylic) [141, 149-152] or sulfonic acid [141, 152], which undergo deprotonation as the environmental pH is above the pKa leading to the ionization of the pendent groups resulting in swelling of the hydrogel. In cationic hydrogels, there is a presence of the cationic pendent groups (amine groups [153]) and swelling of the hydrogel results from an increase in the electrostatic repulsion due to the ionization of pendent group below pKb. Two factors control the degree of swelling of ionic hydrogels; first is the properties of the polymer and other is the properties of the swelling medium [154, 155]. Chitosan, a cationic polyelectrolyte, work well in the low pH environment of the stomach however, the anionic hydrogels such as PAA, polymethacrylic acid, etc. work effectively in the alkaline environment of the colon [58].

The acidic hydrogels (—■—) are ionised in the medium of higher pH, which have an excess of hydroxylic groups. On other hand, basic hydrogels (—●—) swell in solutions of lower pH by protonation of basic groups. The amphiphilic hydrogels (--▲--) contain both acidic and basic groups (Fig. **12**) and thus show two phase transitions [156].
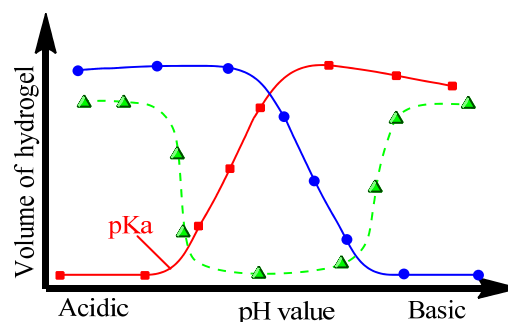

Fig. (12). Phase transition behaviour of polyelectrolyte hydrogels. Modified with permission from ref. [156].
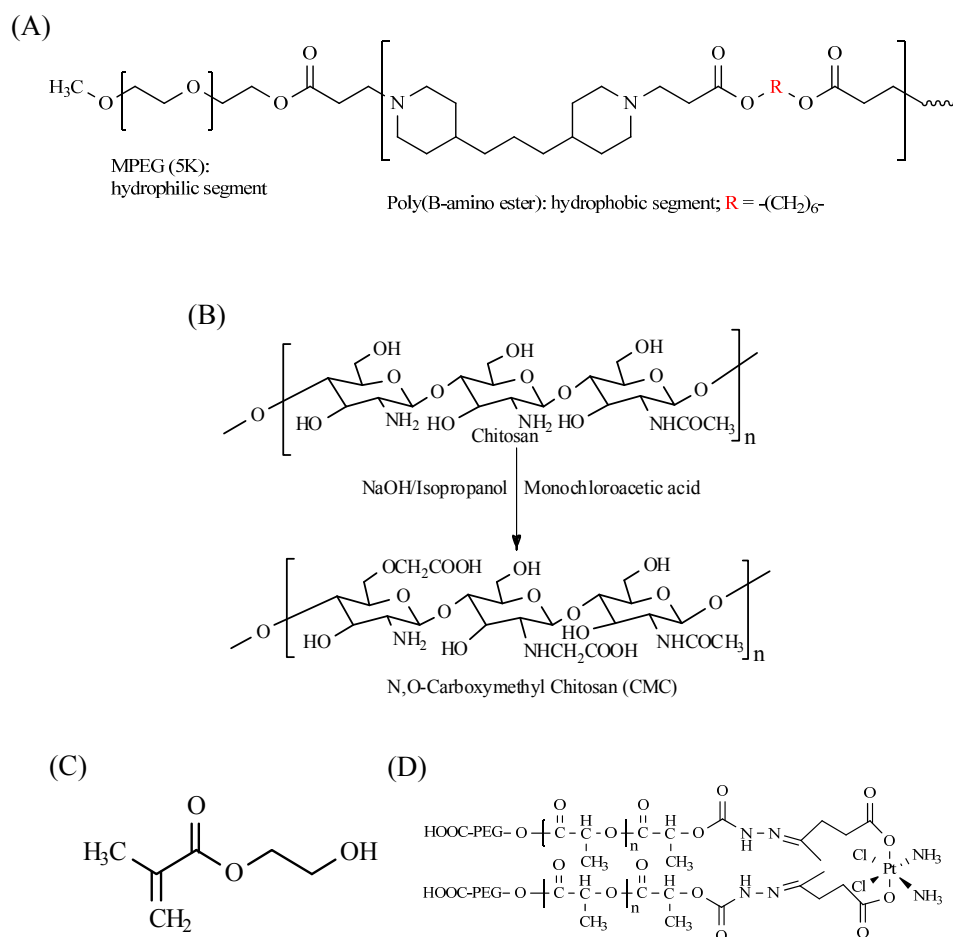
(A)

MPEG (5K):
hydrophilic segment

Poly(B-amino ester): hydrophobic segment; R = -(CH$_2$)$_6$-

(B)

Chitosan

NaOH/Isopropanol | Monochloroacetic acid

N,O-Carboxymethyl Chitosan (CMC)

(C)   (D)

Fig. (13). (**A**) MPEG-poly(β-amino ester) Structure; (**B**) CMC Synthesis; (**C**) HEMA Structure; (**D**) Bi(PEG-PLA)-Pt(IV) Conjugate Structure.

Saito *et al.*, developed PEG-conjugates namely MPEG-DOX and doxorubicin-PEG aldehyde (DOX-PEG$_{CHO}$) and poly(vinyl amine)-PEG-doxorubicin (PVAm-PEG-Doxo) hydrogel crosslinked by PEG-Schiff base linkages. DOX was situated into gel by pendant Schiff base bonds. The release of DOX (due to breakdown of carriers and drug association) was observed to be dependent on pH and ratio of PVAm to the PEG-dialdehyde crosslinker [157].

Lim *et al.*, developed drug delivering magnetic nanoparticles (DMNPs) as theragnostic nano carriers which were sensitive to pH, and release DOX at acidic environment by reduction in the non-covalent association in carriers and drugs complex due to DOX protonation [158]. The combination of hydrophilic methyl ether poly(ethylene glycol) (MPEG) and pH-responsive and biodegradable poly(β-amino ester) afforded amphiphilic pH-responsive and biodegradable polymeric micelle MPEG-poly(β-amino ester) (Fig. **13A**) drug carriers. MPEG-poly(β-amino ester) was efficiently (74.5%) loaded with doxorubicin (DOX) and studied for the release. In an *in vitro* experiment pH-dependent micellization-demicellization behavior of DOX-loaded polymeric micelles was noticed with rapid release at pH 6.4 and slow release at physiological pH (7.4). It was also observed that tumor cells uptake high concentratrion of DOX at pH 6.4 than physiological pH. When DOX-loaded polymeric micelles were injected into B16F10 tumor-bearing mice, significant tumor suppression and prolonged survival of the tumor-bearing mice was recorded as compared to mice treated with free DOX [159].

Deng and co-workes have repoted a pH based reversible gel, which switched into polymer (monomer) solution when subjected to lower acidic conditions while upon the addition of a base it turned back into the gel state. The condensation of bis(acylhydrazine) reversible acylhydrazone bond based dynamic gel was prepared by mixing it with functionalized PEO (A2) and tris[(4-formylphenoxy)methyl]ethane (B3) in organic solvents [160].

Singh *et al.*, developed psyllium and PAA-based hydrogel by the use of radiation-induced crosslinked polymerization and loaded with 5-FU as double potential drug delivery device. Firstly, Psyllium itself is full of therapeutic importance in reducing colon cancer risk and on the other hand it is competent to deliver anticancer agent. These pH responsive hydrogels showed good degree of swelling at pH 7.4 and indicated successful controlled and sustained release of loaded 5-FU into colon through non-fickian diffusion mechanism in which rate of drug diffusion and rate of polymer chain relaxation are comparable (water migration into device and drug diffusion through continuously swelling hydrogel) [161].

Shantha *et al.*, prepared pH responsive hydrogel by the use of *N*-vinyl pyrrolidone (NVP) and poly-(ethylene glycol) diacrylate (PEGDA) and chitosan which show biocompati-

bility and biodegradability for controlled delivery of 5-FU and theophylline. These co-polymeric hydrogels were prepared by a free-radical initiation technique for oral drug delivery. These hydrogels swollen more in simulated gastric fluids (SGF) than simulated intestinal fluids (SIF) and successful *in vitro* delivery of drugs was achieved [162].

Chitosan is a good biodegradable, biocompatible and immunogenic but its applications are limited due to less water solubility at physiological pH [163, 164]. So Chitosan can be turned into water-soluble form carboxymethyl chitosan (CMC) by carboxymethylation (Fig. **13B**) [164]. CMC possesses various characteristics such as biocompatibility, biodegradability, less toxicity and antibacterial activity [165, 166]. Farag and Mohamed found CMC satisfying properties such as pH-sensitive swelling, improved surface property with good antibacterial activity and absorb water up to 500% after 2h [167]. Theophyllin is also delivered using CMC as delivery vehicle and showed sustained release both *in vitro* and *in vivo* [168].

The hydrogels with pH responsiveness and extent of swelling can be fabricated by using neutral co-monomers, for example 2-hydroxyethyl methacrylate (HEMA) (Fig. **13C**) [169]. Xiang *et al.*, synthesized poly(2-hydroxyethyl methacrylate-co-poly(ethylene glycol) methyl ether methacrylate-co-methacrylic acid) [poly(HEMA-PEGMA-MAA)/AT] network based hydrogel in which fibrillar attapulgite (AT) was used as a crosslinker instead of using conventional chemical crosslinker by free radical polymerization process. These hydrogel showed greater equilibrium swelling ratio, improved tensile mechanical properties and faster response rate to pH [170]. A novel acid-responsive CDDP containing Bi(PEG-PLA)-Pt(IV) polymer (Fig. **13D**) prodrug conjugate nanoparticles (NPs) was synthesized by pH sensitive hydrazone bond which covalently links cisplatin analogue prodrug Pt(IV) to the hydrophobic segment of two PEG-Poly-(lactide) (PLA) copolymer chains. The resulting NPs show excellent acid-responsive drug release kinetics with drug burst at pH 5.0 and 6.0 due to hydrazone bond cleavage. Besides, it also showed well-controlled cisplatin loading yield with cytotoxicity against A2780 human ovarian carcinoma cell line *in vitro* [171]. The dendronized heparin-DOX conjugates (heparin-DOX) have been developed as pH sensitive drug delivery system (Fig. **14**). It was observed that these conjugates self-assembled with negatively charged surface into a compact nanoparticle. These nanoparticles showed significant anti-tumor activity, high anti-angiogenesis and also induced apoptosis when applied to 4T1 breast tumor model in mice with faster drug release rate at pH 5.0 without being toxic to healthy tissues/organs [172].

Curcumin (CUR) extracted from herb *Curcuma longa* also known as turmeric even it can also be derived from ginger root is well-known for anti-cancer properties [173-177]. However, CUR has limited efficacy due to poor aqueous solubility, minimum systemic bioavailability, degradation in light and at high pH. Deepa *et al.*, prepared CUR incorporated hydrogel which is PEG cross-linked acrylic polymers (PEGDA-PAA) (Fig. **15A**) loaded with hydrophobic CUR as model drug. The entrapment efficiency of 0.5% and 1% cross-linked polymers was recorded as 71.6% ± 1.6% and 67.5% ± 0.51%, respectively. It was noticed that the entrapment efficiency decreased as cross-linking increased, which

might be due to differences in the swelling behaviour. Nanogel (0.5% and 1%) showed swelling of 36.6 ± 0.82 g/g and 21.2 ± 1.34 g/g of nanogel at pH 7.4, respectively. Nanogel with 1% crosslinked polymer showed reduced swelling than 0.5% because of high cross-linking due to which there is sustained release of loaded drug in case of 1% nanogel. Curcumin nanogels (0.5% and 1%) induced caspase 3 and caspase 9 more effectively than free CUR in dimethyl sulfoxide (DMSO). It was observed that CUR nanogels show more cytotoxicity and apoptotic effects toward cancer cells than free CUR as assessed by caspases 3 and 9 in HeLa cells [178].

Singh and Bala explored poly(acrylamide-co-acrylic acid) crosslinked with psyllium [psy-cl-poly(AAm-co-AAc)] hydrogels for sustained release of methotrexate from hydrogels in the colon. The hydrogels were noticed to have higher swelling properties in the pH 7.4 which was attributed to pH responsiveness of pAAm and pAAc. These hydrogels also have shown high swelling phenomenon in NaCl salt solution (5.91 ± 0.17 g/g of gel) with increase in temperature upto 37 °C. The release of methatrexate was observed more at pH 7.4 which indicated its colon specificity for drug release and also due to bioadhesive property towards mucosal layer of small intestine [179].

5.3. Drug Release Regulated by Biological Stimulus

There are various biomolecules like enzymes, antigens within organism that possess strength to drive drug release system of from smart hydrogels come under biological stimulus sensitive hydrogel.

5.3.1. Hydrogels Depend Upon Enzyme Substrates for Drug Release

A well known anticancer drug, cisplatin (CDDP) is a widely used for the treatment of cancer. Unfortunately, cisplatin fails to enter to the cell as it bounds to the plasma proteins in the blood [180]. In order to enhance the specificity of CDDP, hydrogels were employed as target drug delivery vehicles. For example glioblastoma multiforme is related with the overactive matrix metalloprotenase (MMP). Tauro *et al.*, developed a sophisticated system possessing MMP complexed with CDDP was incorporated into PEGDA hydrogel (Fig. **15B**) wafers having different PEG chain lengths ($M_n \approx 574$ and 4000). The release of active CDDP depends upon the cleavage of MMP substrate and drug association. It was observed that hydrogel with chain length of 4000 monomers shows substantial increase in the drug release, which provides a space to MMP activity in the hydrogel network. The bioactivity of CDDP also gave specificity as hydrogel spiked with MMP substrates only target glioblastoma multiforme. It was noticed that PEGDA hydrogels and PEGDA-peptide hydrogels without CDDP were nontoxic and not influenced by the presence or absence of MMP-2 or MMP-9. Thus, this hydrogel can be considered for the controlled delivery of active cisplatin to tumor tissues with significant specificity [181-183]. Taxol is a well-known effective anti-cancer drug that arrests mitosis and drives apoptosis. However, the biological applications of taxol are limited due to its hydrophobic functionalities present in the structure of same. In order to overcome this problem, the target delivery of taxol was considered as strong tool [184]. Xu *et al.*,
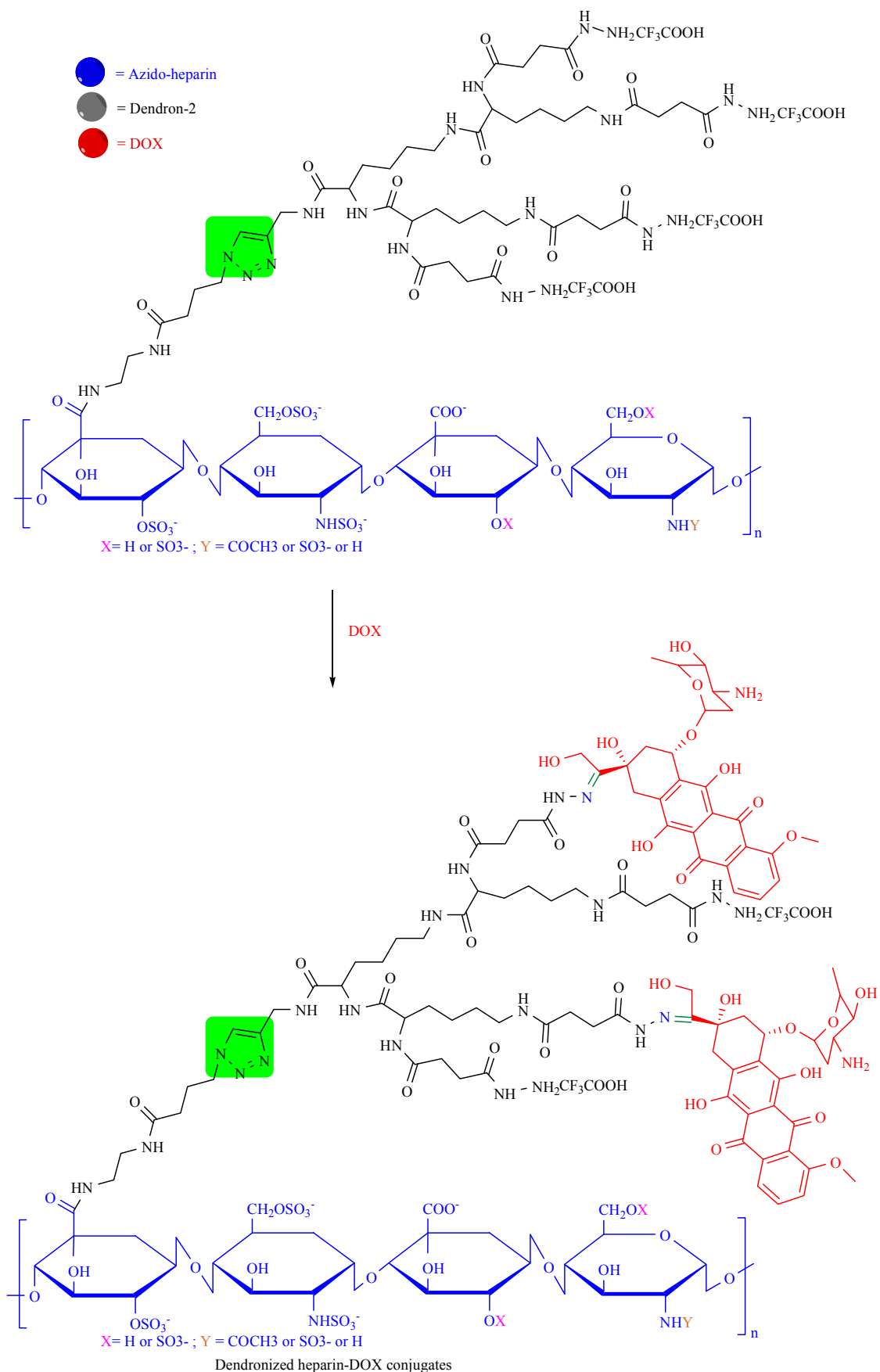
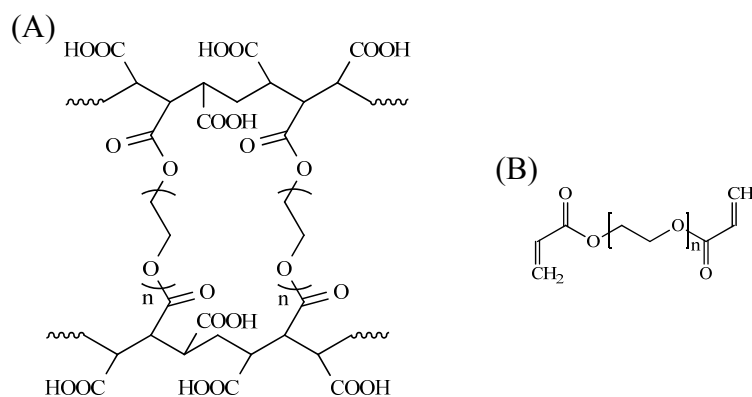**Fig. (14).** Dendronized Heparin-DOX Conjugate Structure.

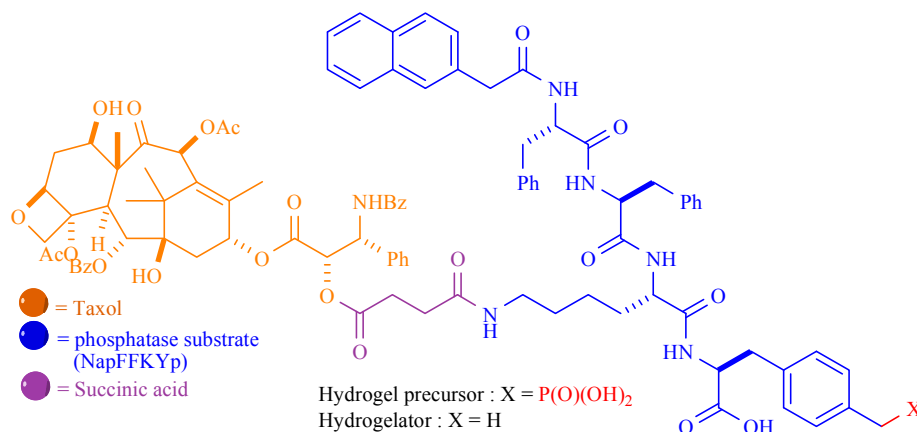**Fig. (15). (A)** PEGDA-PAA Polymer Structure; **(B)** PEGDA Structure.

Fig. (16). Taxol based hydrogel precursor and hydrogelator structure.

developed taxol based hydrogel precursor which turned into hydrogelator by enzymatic (Phosphatase cleavage) action and self-assembled into nanofiber and ultimately form supramolecular hydrogel without compromising cytotoxicity of the taxol on HeLa cells. This supramolecular hydrogel was observed to release hydrogelator in sustained manner into aqueous environment. It must be noted that these nanofibers possess dual role one as drug delivery system and as drug itself for chemotherapy. Taxol based hydrogel precursors are shown in Fig. **16** [185].

In 1998, Duncan and co-workers developed the first *N*-(2-hydroxypropyl)methacrylamide (HPMA) polymer-DOX conjugate, Prague-Keele-1 (PK1) [186, 187]. Since these conjugates have the preclinical experiments, it was suggested to proceed with phase1 clinical trials. In PK1, DOX is linked with HPMA by a peptidyl linker [186] and PK1 releases DOX when peptide linker is cleaved by lysosomal cysteine proteinases inside tumor [188, 189]. HPMA is water soluble, biocompatible but non-biodegradable possessing a large number of the pendent functional groups that allow the conjugation of hydrophobic anticancer drug to the polymer backbone *via* an enzymatically degradable linker (usually Gly-Phe-Leu-Gly) [190].

5.3.2. Specific Antigen-responsive Hydrogels

The controlled drug release in response to specific "signalling molecules" released as a product of the immune response or by any foreign invaders leads to the development of antigen (Ag) sensitive hydrogels. In antigen sensitive hy-

drogel, antigen and antibody (Ab) are immobilized in the monomer chain and are cross-linked making hydrogel in deswelling state but in presence of free antigen these cross-links reduced due to antibody interaction towards free antigen which leads to hydrogel swelling [191, 192]. The Ab-Ag cross-link dissolution results in the release of drug trapped inside [141]. The fabrication of smart hydrogels enables control drug release, which are responsive to signalling molecules released as a product of immune response or by a foreign invader.

In 2003, Lu *et al.*, reported issues related to low sensitivity of hydrogel to Ag due to non-specificity in chemical conjugation of Ab at ε-amino group of lysine and the problem was tackled by conjugation of a monomer to cysteine residues to make polymerizable Ab Fab' (antigen binding fragment) fragments [193]. A new polymerizable Fab' fragment, BDC1 (IgG2a) was fabricated from anti-fluorescein monoclonal antibody and was used for the hydrogel preparation with NIPAAm and MBA (Fig. **17**). Hydrogel with low Fab' contents (FPN-1) and a high Fab' content (FPN-2) were prepared. It was observed that temperature for sharp volume change increased with increase in Fab' contents *i.e.* higher the Fab' contents, smaller the magnitude of volume change. These hydrogels were tested with free fluorescein (FL) and a fluorescein-polyamidoamine (PAMAM) dendrimer conjugate (FD) in acetate buffer (10 mM, pH 5.0) for their antigen responsiveness at three different temperatures. The results indicated significant volume changes were observed at high temperature for FPN-2 hydrogel particularly, when the Ag
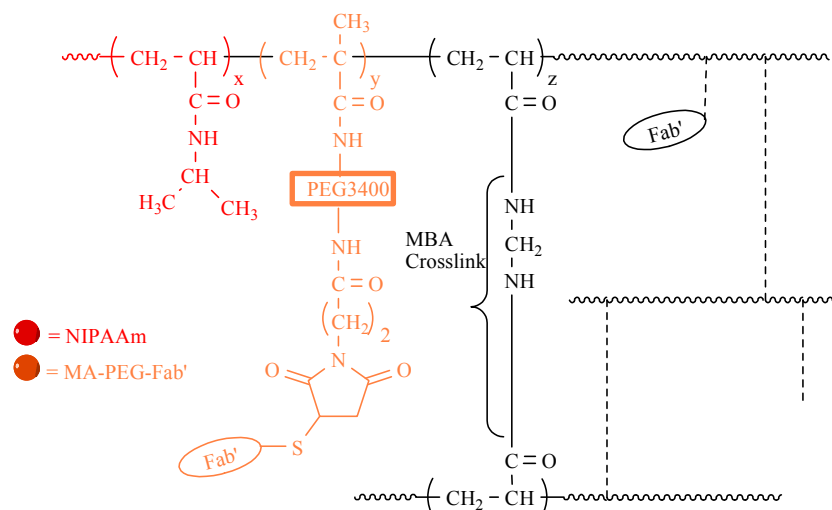
Fig. (17). Fab' fragment based hydrogel Structure.

were switched *i.e.*, gel shrinked in FL solution and recovered its volume in FD solution. Thus it was concluded that Ag responsive swelling property depends on the Fab' contents of the hydrogel. It was also observed that FPN-2 gel shows better response at high temperature and near the low pH range especially at pH 5 [193]. There are some other Ab fragments like single chain variable fragments (scFv) and third generation (3G) having multi-specificity and conjugation with exogenous functional moieties [194] so this function is need to explore in terms of hydrogel.

In 1999, Lu *et al.*, also worked on the preparation of targeted systems by co-polymerization of polymerizable antibody Fab' fragments, HPMA and drug-containing monomers. The resulting polymers showed high cytotoxicity against OVCAR-3 human ovarian carcinoma cells with the loaded photodynamic anticancer drug Mce6 [195]. This technique might be useful in the design of hydrogel sensitive to various tumor specific antigens such as alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA) etc. [196].

Casey and Kofinas reported carcinoembryonic antigen (CEA) imprinted poly(allylamine hydrochloride) (PAlAm-HCl) hydrogel where ethylene glycol diglycidyl ether (EGDE) is a water soluble crosslinker The positive charged residues in the polymer electrostatically and specifically binds to the negatively charged amino acids of the CEA protein creating cavities with high affinity for target molecules *i.e.* CEA on adenocarcinomas target cell surface due to its intercellular homotypic adhesion molecule character. The specificity of imprinted hydrogel towards CEA was studied by its attachment with AFP which is structurally and electrostatically similar to CEA. Results show CEA imprinted poly(allylamine hydrochloride) (PAlAm-HCl) hydrogel has more affinity to CEA with imprinting factor 5 for CEA and 2 for AFP. When using control nonimprinted hydrogel, slightly more AFP is adsorbed than CEA which ultimately concluding more selectivity of imprinted hydrogel towards CEA [197]. These characteristics can be used for drug delivery also.

Prostate-specific antigen (PSA) is a specific protease secreted by prostate cancer cells. Ikeda *et al.* has reported functional supramolecular hydrogel capsule 1/2 (SH capsule 1/2)

made from supramolecular hydrogel comprising glycolipid mimic 1 and PSA labile additive 2. PSA labile additive 2 contains a PSA specific peptide substrate, DUPA (2-[3-(1,3-dicarboxypropyl)ureido]pentanedioic acid) to target the prostate cancer cells (PCa), TAMRA as drug model and pyrene moiety to link PSA substrate to supramolecular fiber [198]. It was noticed that when PSA diffuses into the SH capsule 1 it causes proteolytic cleavage of PSA labile additive 2 resulting to the release of cleavage product 3, a hydrophilic fragment (Fig. **18**). In these experiments tetramethylrhodamine (TAMRA) was used as a drug model and its localization and amount of 2 and 3 were studied by both confocal laser scanning microscopy (CLSM) observation and fluorescence spectroscopy measurement. For targeting purposes, prostate-specific membrane antigen (PSMA) was used which is a plasma membrane associated glycoprotein over-expressed on PCa cells and known to get internalised through clathrin coated pits and hence considered as a suitable drug carrier for PCa cells.

5.3.3. Receptor Mediated Delivery of the Immunotherapeutic Agents

Schenk *et al.* developed a specific poly(2-oxazoline) based hydrogel network with incorporation of RGD peptide [199]. The tumor cells show overexpression as well as high activity of integrins specifically $\alpha_v\beta_5$ integrin and its congener $\alpha_v\beta_3$. The cyclic RGD motif shows strong interaction with these integrins. However, it required to explore the use of this hydrogel for cancer drug delivery to accomplish enhanced therapeutic value.

6. DRUG RELEASE REGULATED BY BOTH PHYSICAL AND CHEMICAL STIMULI: DOUBLE NETWORK HYDROGELS

Before 1994, hydrogel with single network of polymer, responsive to single stimulus (such as either temperature or pH) used to consider as suitable vehicles for the treatment of cancer. In 1994, Gong and co-workers brought the new concept of double network hydrogels that are sensitive to two stimuli in single time [46]. The double network hydrogels were prepared by the chemical cross-linking of two independent
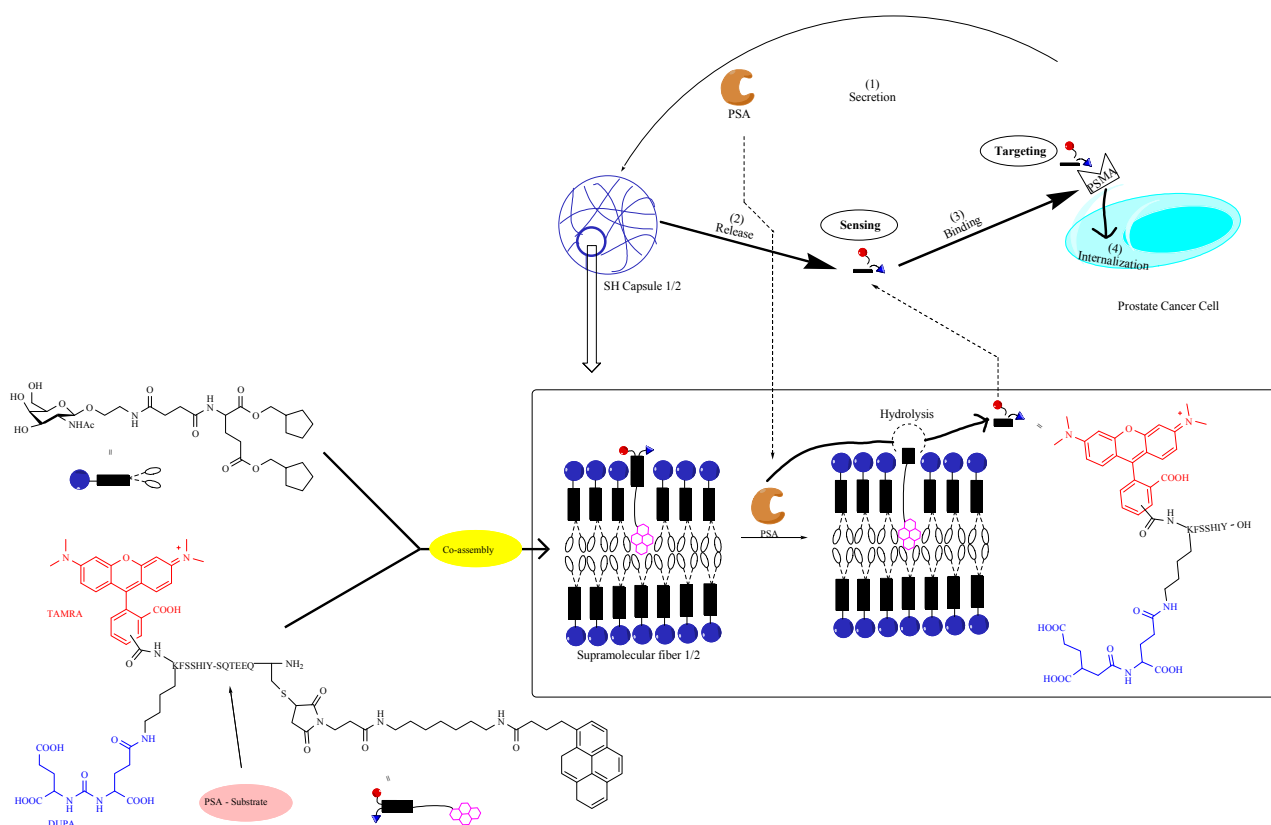
Fig. (18). PSA sensitive hydrogel synthesis and action mechanism. Modified with permission from ref. [198].

networks *viz.* ionisable and neutral network. The double network smart hydrogels, sensitive to temperature and pH have come into lime light recently. Li *et al.*, successfully prepared and characterised the double network hydrogels using PNIPAAm as a tightly cross-linked (ionizable 1st network) and PAA as loosely cross-linked (neutral 2nd network) with graphene oxide (GO) as an additive. FT-IR spectrum of PNIPAAm/AAc/GO hydrogel demonstrated a new adsorption at 1724 cm^{-1} which was assigned to -COOH of AAc. These residual unreacted -COOH group result in pH sensitivity of the double network hydrogel. The analysis of SEM advocated the largest pore size was attributed to PNIPAAm/GO2 hydrogel which is influenced by crosslink density of the hydrogel. These results suggested that the swelling ratio and the response rate of the hydrogel influenced by AAc constituents. Differential scanning calorimeter (DSC) indicated that volume phase transition temperature (VPTT) of the hydrogel is decreased with an increase in the contents of GO sheets. These terrific thermal conduction of GO impart temperature responsiveness to the double network hydrogel [200].

A successful example of crosslinked polymeric micelles, sensitive to pH and light was developed from self-assembly of amphiphilic glycol chitosan-o-nitrobenzyl succinate conjugates (GC-NBSCs). The cytotoxicity and drug release study of these conjugates were performed upon loading with CPT. The experiments (*in vitro*) showed the quick drug release at low pH with the light irradiation against NIH/3T3 cells and under UV irradiation displayed good cytotoxicity against MCF-7 cancer cells [201].

Ta *et al.*, prepared a temperature and pH responsive chitosan hydrogel with dipotassium orthophosphate as gelling agent and this hydrogel was used for sustained release of DOX and anti-cancer gene (pigment epithelium-derived factor plasmid) to the tumor site. The results showed that co-delivery of both chemotherapeutic drug and anticancer gene efficiently suppresses tumor growth without any side effect [202].

7. HYDROGEL WITH STRUCTURAL MODIFICATIONS

7.1. Small Peptide based Molecular Self-Assembly: Nano Hydrogels

Peptide-based self-assemblies are under great attention because of ample of desire characteristics such as their low cost, specific molecular recognition, easy tailoring of their chemical and biological functionalities and also their biomimetic nature [26, 203]. Peptide based small molecules have been explored as nano hydrogels possessing numerous applications in drug delivery systems [204]. These low-molecular-mass, biocompatible, non-toxic biodegradable building blocks have the ability to self-assemble into well-ordered hydrogel that can be utilized towards medical applications [43]. Natural (α-helix and β-sheet) and synthetic (peptide amphiphiles, π-stacking systems) peptides have been used in self-assembling nanoparticle or nano hydrogel formation [44]. The charge distribution on peptides and their conformations influence their assembly and gelation, respectively [205, 206]. In the peptide skeleton, sequences with more of hydrophobic residues show lower critical self-assembly concentration (CSAC) or critical aggregation con-
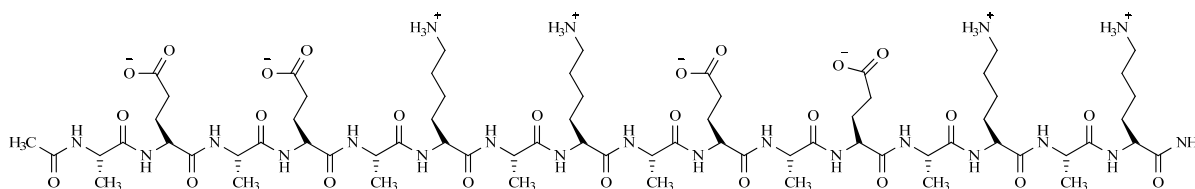
Fig. (19). EAK16-II peptide structure.

centration [207]. The peptide, EAK16-II (AEAEAKAKAE AEAKAK) (Fig. **19**) has been explored to study the effect of salt to mask the charges in the peptide and it was noticed that the CSAC is not affected significantly by NaCl. The salt promotes assembly of fibres as it allows the desired interactions and prevents random interaction at low concentration however; the effect of salt is reduced with an increase in the concentration of peptide [208]. Wu *et al.* took one step ahead and used EAK16-II as drug delivery carrier for ellipticine (EPT). It was found that the self-assembled peptides significantly stabilize (in aqueous solution) EPT in terms of hydrophobicity and toxicity. The encapsulated EPT in EAK16-II was studied for cellular uptake, toxicity, and apoptosis in A549 human lung carcinoma cell line (*in vitro*). It was found that EAK-EPT complexes were significantly effective than EAK16-II or EPT alone in A549 nude mouse tumor model (*in vivo*). It was also noticed that EAK-EPT complex had high antitumor activity and lower cytotoxicity than ellipticine alone [209].

Liu *et al.*, developed PTX containing self-assembling peptide RADA16 hydrogel (RADA 16-PTX hydrogel) with significant inhibitory impact on breast cancer cell line (MDA-MB-435S) growth [210]. An increased concentration of peptide decreased drug release rates *i.e.* longer released $t_{1/2}$ which ultimately enhanced efficiency of therapy by increasing the drug contact time at action site. E. Gazit and co-workers have prepared a new peptide based hydrogel consisting of N-fluorenylmethoxycarbonyl-di-phenylalanine (Fmoc-FF) core, and vitamin E-$_D$-α-tocopheryl polyethylene glycol 1000 succinate (vitamin E-TPGS) monolayer as an outer shell by inverse emulsion technique utilizing the ability of Fmoc-FF building block to form hydrogels *via* assembly in water without the need of cross-linking or any other covalent bonding [43]. The newly prepared hydrogel matrix was utilized to encapsulate anti-cancer drugs such as DOX and 5-FU and found that encapsulation ability of hydrogel nanoparticles depends on the physicochemical characteristics of the encapsulated molecules which affect their release kinetics. It was demonstrated experimentally that these hydrogels can be used to deliver various drugs effectively.

A peptide-polysaccharide hybrid hydrogel possessing Fmoc-FF as β-sheet peptide and konjac glucomannan (KGM) was prepared which was highly hydrated, rigid and showed high mechanical strength. This hybrid hydrogel was studied for the *in vitro* delivery of DTX. It was noticed that the variability of KGM concentration, molecular weight, aging time or β-mannanase concentration afforded the sustained and controlled drug release [211].

7.2. DNA Based Hydrogel

A few polymers, sensitive to pH, temperature or both are available and known for their successful biomedical applica-

tions which could be reasoned to the constant or least fluctuating human physiological internal environment. To resolve this problem more flexible and biocompatible hydrogels have been designed and explored for their applications [212]. The employment of aptamers for the fabrication of hydrogels is an emerging research for the treatment of cancer. The aptamers are single-stranded DNA molecules, an inherent biocompatible specialize to specifically recognized targets which range from cells to ions even at low concentration and undergo structural changes upon binding that makes them suitable for *in vivo* application. Moreover, their high affinity, high avidity, easy modification and fabrication and low cost make them effective model for hydrogel tailoring. The structural changes of adapters could be a milestone for their applications in the treatment of cancer as the structural changes result in the dissociation of hydrogel structure which leads to drug release [213].

7.3. Lipogels

Liposomes, firstly defined by Bangham and co-workers [214] are being used in various biomedical fields like diagnostic imaging, gene delivery, photodynamic therapy, vaccine adjuvant, hemoglobin or chelating agent transporter and enzyme replacement therapy [215-219]. Liposomes are well established drug carriers which are also used for specificity in cancer treatment [220]. The therapeutic agent is generally incorporated into the lipid bilayer and/or the lumen with the possibility to fabricate drug release mechanism *via* choice of lipids [218]. Jensen *et al.* reported the assembled lipogels that are composites of hydrogel assembled from PVA and liposomes (Fig. **20A**) [221]. PVA hydrogels in its pure form are not well suited for cargo immobilization and thus the material leaks out with a burst effect. In order to test the drug delivery efficiency of composite hydrogel, liposomes were incorporated into PVA hydrogels through thiol-disulfide exchange between thiocholesterol containing liposome and end group modified PVA. These lipogels do not support adhesion and proliferation of mammalian cells due to its PVA components. To make these matrices cell adhesive, two strategies, the blending PVA with a cationic polypeptide poly-($_L$-lysine) (PLL) and coating with poly(dopamine) were employed. It was observed that lipogels with PLL show high cell attachment which ultimately results in transfer of paclitaxel to the surrounding cells and decrease in cell viability.

An injectable DOX-LP/HTCC-GP gel system was developed *in situ* by the combination of DOX-loaded liposomes (DOX-LP) and themosensitive hydrogel (HTCC-GP) based on quaternary chitosan salt, N-[(2-hydroxy-3-trimethyl-ammonium)propyl] chitosan chloride (HTCC), and Glycerophosphate (GP). DOX-LP showed reduced side effects with slight compromise in antitumor activity. The improved antitumor activity was achieved for HTCC-GP/ DOX-LP with
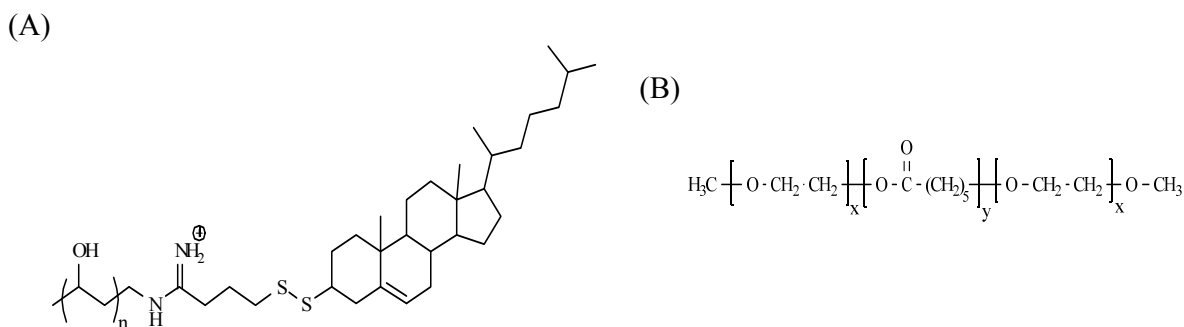
(A)

(B)

Fig. (20). (**A**) Lipogel Structure; (**B**) The chemical structure of PEG-PCL-PEG.
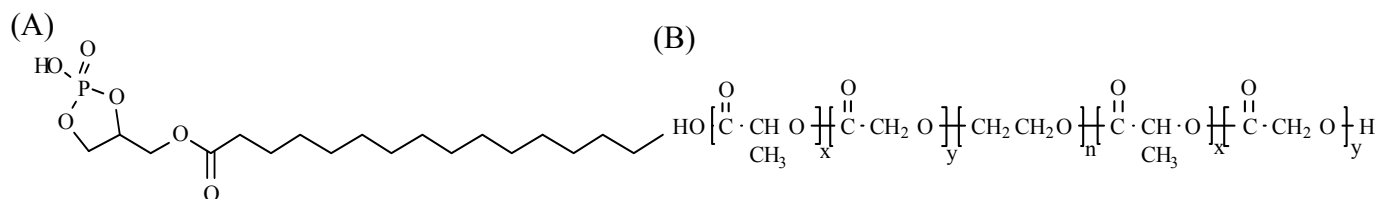
(A)

(B)

Fig. (21). (**A**). cPA structure; (**B**). PLGA-PEG-PLGA tri block polymer structure.

mean survival time (MST) of 35 days and 20% ICR mice (intraperitoneally inoculated with H22 cells) survived for above 60 days [222].

8. BIODEGRADABLE POLYMERS

Biodegradable polymers are capable of cleaved into bio-compatible side products *via* enzyme-catalyzed or chemical hydrolysis irrespective of their origin *i.e.* natural or synthetic. This property of makes them an ideal tool to implant into the body without subsequent surgical removal [223]. The chemical reactions such as polymeric chain breakage caused by the hydrolytic or enzymatic degradation in the hydrogel matrix determine the biodegradability of the hydrogel system [224]. Eventually, use of hydrogel systems in the treatment of cancer could provide multi-modality therapies with more efficacies over therapies with low or poor survival rates.

Wang *et al.*, prepared biodegradable and temperature sensitive PEG-PCL-PEG (PECE) (Fig. **20B**) triblock polymer/hydrogel for loading 5-FU. Initially, a blank PECE was prepared followed by 5-FU loaded and 5-FU drug release profile of PECE hydrogel was studied under phosphate buffer solution (PBS, pH 7.4), a release medium. Hydrogel (PECE-5-FU) loaded with lower initial 5-FU drug amount (0.5 mg) showed high cumulative release rate (95.3%) than high initial 5-FU drug (1.0 mg) loaded hydrogel (84.6%). It was found that 5-Fu hydrogel showed significant effectiveness with poor hematologic toxicity [225].

A biodegradable photo-crosslinked dextran (DEX) hydrogel incorporated with cationic linear polyethyleneimine (LPEI) and ester linkages was prepared and explored for biomedical applications. LPEI is electrostatically linked with siRNA within hydrogels. LPEI/siRNA is released when ester linkage between LPEI and DEX hydrogel degraded. A low cytotoxic effect was observed against human embryonic kidney 293 cells (HEK293). The release rate of siRNA was ob-

served to be influenced with DEX and LPEI concentrations. It was demonstrated that these hydrogels can be used for sustained release of siRNA to treat cancer with high specificity and low toxicity to normal surrounding cells [226].

Cyclic phosphatidic acid (cPA) (Fig. **21A**) is known to possess growth inhibitory activity against colon cancer cells as it inhibits peroxisome proliferator-activated receptor gamma (PPARγ) pathway [227]. However, lysophosphatidic acid (LPA) is formed when cPA undergoes hydrolytic cleavage by lipid phosphate phosphatases that has growth stimulatory activity [228]. Tsukahara *et al.*, synthesized cPA possessing gelatin (natural, biodegradable, non-toxic macromolecule) based hydrogel microsphere. It was found that hydrogel reduces the hydrolytic cleavage of cPA to significant level than cPA alone so that growth of HT-29 cells is reduced. Also, the treatment with cPA hydrogel efficiently suppresses LPA-induced PPARγ activity, HT-29 cell proliferation and migration and hence cPA hydrogel might be very helpful for the treatment of cancer [228].

PEG based hydrogel such as PLGA-PEG-PLGA (Fig. **21B**) are resistant to biodegradation. Therefore, surgical removal is required that ultimately increases the treatment cost and patients also suffer from unnecessary surgical procedures. Synthetic polymers are also not very much compatible to all extent. Seib *et al.,* prepared a biodegradable and biocompatible self-assembling non-swellable silk hydrogel loaded with DOX [229]. It was found that silk-DOX hydrogel possesses significant inhibitory impact on relapse disease.

PLGA and poly-(lactic acid) (PLcA) were employed to prepare a biodegradable microspheres from leuprorelin acetate (LpA) entrapped in PLGA based micelle [15, 16, 230, 231]. LpA is a synthetic nonapeptide GnRH analogue which has longer half-life and 80 times more potent than natural occuring GnRH. It was launched first time in 1985 for the treatment of prostate cancer because it acted as an inhibitor
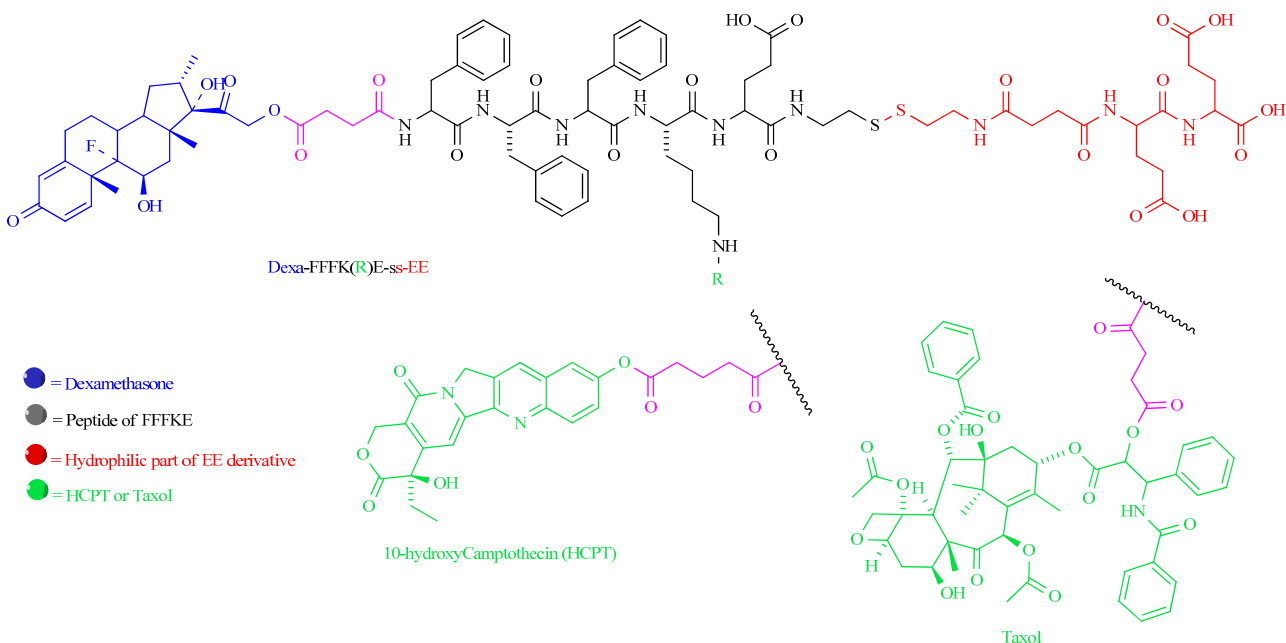
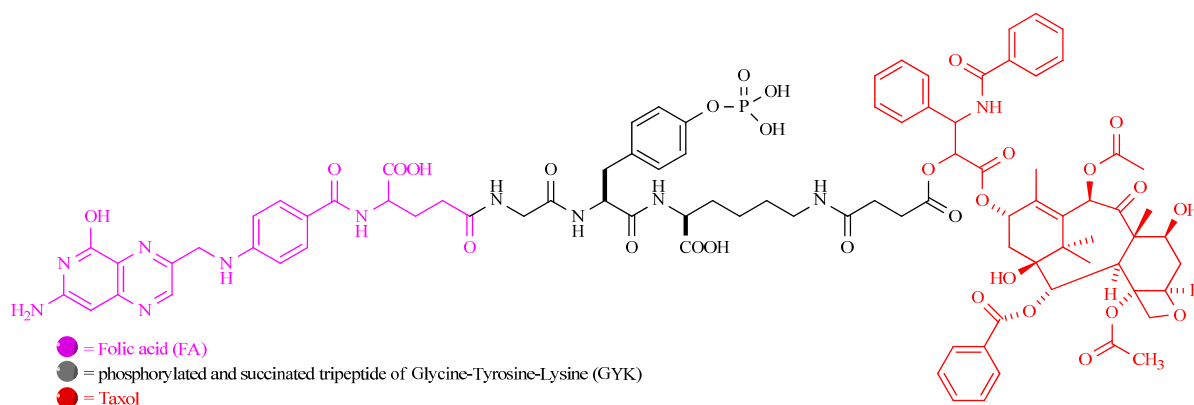**Fig. (22).** Dexa-FFFK(HCPT/Taxol)E-ss-EE hydrogel structure.

Fig. (23). FA-GpYK-Taxol nanosphere structure.

of gonadotropin and suppresses ovarian and testicular steroidogenesis [231, 232]. The sustained release (3 months) of LpA from PLGA-PLA hydrogel resulted in steroidogenesis suppression while LpA released from PLcA matrix is limited for 1 month. This conjugate is helpful in prostate cancer and endometriosis therapy. Musumeci *et al.*, also prepared the biodegradable nanosphere colloidal suspensions from PLA and PLGA and used for intravenously administration of DTX [233]. The biodegradable thermo-responsive star-shaped block copolymer (4arm PLGA-PEG) solutions were loaded with doxorubicin (DOX) and showed significant tumor inhibition in KB tumor cells bearing CD-1 mice [234].

9. CONJUGATION OF COMPLEMENTARY ANTI-CANCER DRUGS CONFERS MOLECULAR HYDROGELS AS A CO-DELIVERY SYSTEM

The limitations of chemotherapy can be eliminated by the exposure of patients to more than one anti-cancer agents. In the current scenario, the liberation of two complementary anti-cancer drugs entrapped in a single molecular hydrogela-

tor remains the most challenging step. Mao and co-workers reported a molecular hydrogel system based on two complementary anticancer drugs (taxol and cis-platinum-based drugs) for chemotherapy [235]. They designed and prepared dexa-FFFK(Taxol/HCPT)E-ss-EE as a precursor of molecular hydrogelators composed of dexamethasone (Dexa), amino acids (F, K, E), taxol and 10-hydroxy camptothecin (HCPT). Tgel (Taxol derivative) and Hgel (HCPT derivative) were mixed at different ratios for testing the possibility of hydrogel system for delivery of two kinds of chemotherapeutic drugs along with the release profile of Taxol and HCPT at 37°C. The structure of taxol and HCPT is shown in Fig. **22**. A similar similar release profiles were observed for taxol and HCPT independently. In clinics, liposome, conjugates of anticancer drugs and hydrophilic polymer or proteins, nanocapsules fabricated from block polymer and polymeric hydrogels are very promising candidates for the development of cancer therapy [235]. In another example, a self-assemble nanosphere molecular hydrogelator was loaded with taxol and the tumor targeting folic acid and was employed for drug delivery (Fig. **23**) [236].

10. CONCLUSIONS AND FUTURE DIRECTIONS

Although several anti-metastasis drugs have been isolated but their administration is limited. Hydrogels have been discovered with their capability to deliver drug efficiently irrespective of the hydrophobic nature of the drug with significant specificity to target sites with reduced cytotoxic effects. Hydrogel based nanocarriers have been utilised to study pharmacokinetics and pharmacodynamics of various drugs both in *in vitro* and *in vivo* system. Hydrogel hierarchy shows that how hydrogel controlled drug delivery system developed from synthetic polymer to the DNA based polymer *via* peptide based polymer. After detailed analysis of smart hydrogel in the direction of nano drug carrier, it would not be wrong to say that hydrogel has a bright future and could be a great hope for cancer therapy. Scientists are trying to develop more biocompatible drug vehicle and DNA based hydrogel is a results of their extreme efforts. So there is an urgent need of more research to be carried out on biodegradable, biocompatible nano-sized hydrogelators with good mechanical strength, and rapid actions to deliver better treatment against cancer related problems.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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