Future Journal of Pharmaceutical Sciences

REVIEW Open Access

Pharmaceutical applications of citric acid

Sopan Nangare[1], Yogini Vispute[2], Rahul Tade[1], Shailesh Dugam[3] and Pravin Patil[1*]

Abstract

Background: Citric acid (CA) is a universal plant and animal-metabolism intermediate. It is a commodity chemical processed and widely used around the world as an excellent pharmaceutical excipient. Notably, CA is offering assorted significant properties viz. biodegradability, biocompatibility, hydrophilicity, safety, etc. Therefore, CA is broadly employed in many sectors including foodstuffs, beverages, pharmaceuticals, nutraceuticals, and cosmetics as a flavoring agent, sequestering agent, buffering agent, etc. From the beginning, CA is a regular ingredient for cosmetic pH-adjustment and as a metallic ion chelator in antioxidant systems. In addition, it is used to improve the taste of pharmaceuticals such as syrups, solutions, elixirs, etc. Furthermore, free CA is also employed as an acidulant in mild astringent preparations.

Main text: In essence, it is estimated that the functionality present in CA provides excellent assets in pharmaceutical applications such as cross-linking, release-modifying capacity, interaction with molecules, capping and coating agent, branched polymer nanoconjugates, gas generating agent, etc. Mainly, the center of attention of the review is to deliver an impression of the CA-based pharmaceutical applications.

Conclusion: In conclusion, CA is reconnoitered for multiple novels pharmaceutical and biomedical/applications including as a green crosslinker, release modifier, monomer/branched polymer, capping and coating agent, novel disintegrant, absorption enhancer, etc. In the future, CA can be utilized as an excellent substitute for pharmaceutical and biomedical applications.

Keywords: Citric acid, Pharmaceutical applications, green crosslinkers, Fluorescent materials, Absorption enhancer

Background

Citric acid (CA, 2-hydroxy2, 3-propanetricarboxylic acid, tricarboxylic acid) is the largest organic acid contained in the tonnage. Generally, it is a universal plant-and animal-metabolism intermediate. CA is a commodity chemical processed and widely used around the world for plentiful pharmaceutical applications (Fig. 1) [1]. To begin with 1784, Carl Scheele (a Swedish chemist) isolated the CA (Molecular Weight: 210.14 Da) from the lemon juice. Whereas in1893, at the first time Wehmer demonstrated the culture medium includes sugars and inorganic salts, *Penicillium glaucum* (*Citromyces*) accumulating CA. Amusingly, CA was first commercially manufactured in England from the imported Italian lemons. In 1917, Currie discovered that some of the *Aspergillus niger* strain generated CA into adequate nutrient mediums that contain high levels of sugar plus mineral salts and along with that preliminary medium pH (2.5–3.5). Despite these notable findings, lemon juice was still a commercial source for the manufacturing of CA until 1919. This provided the foundation for industrial CA production with *Aspergillus niger* [2]. As per literature, CA has been unrevealed by Krebs in the late 1930s as a key ingredient in the metabolism of all aerobe species [3, 4]. The developmental stages of the discovery and manufacture of CA from 1784 to 2020 [4] are represented in Fig. 2.

From its inception, plenty of literature reported that CA is a major component in the processing of several products, mainly as an acidulant in the food, chemical, and pharmaceutical industries. Natural resources, such as fruit sugar, become more and more essential for CA

* Correspondence: rxpatilpravin@yahoo.co.in
[1]Department of Pharmaceutical Chemistry, H. R. Patel Institute of Pharmaceutical Education and Research, Shirpur, Dist-Dhule 425405, India
Full list of author information is available at the end of the article

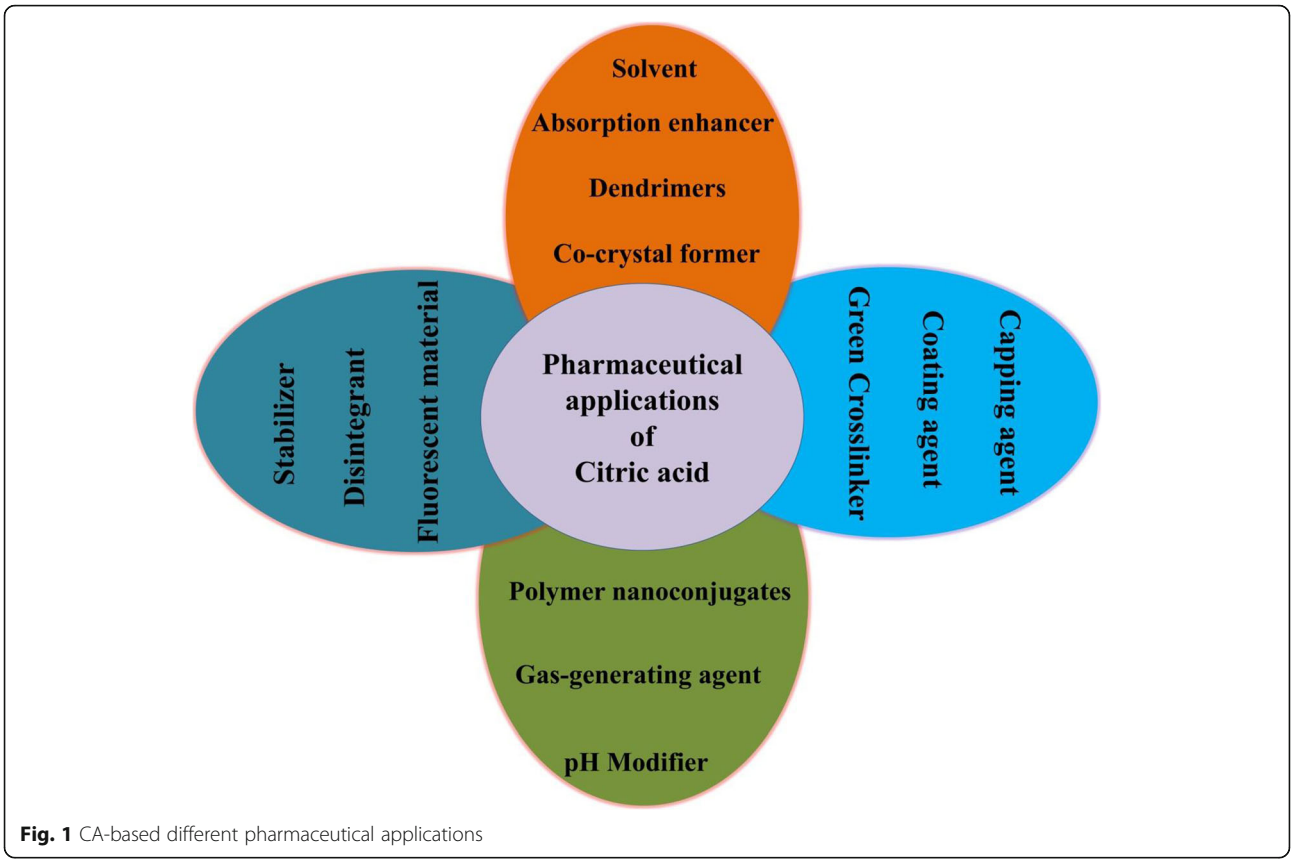

Fig. 1 CA-based different pharmaceutical applications

processing, giving the foods and beverages a good, delicate flavor. They are also referred to as additive, medicinal, cosmetic, and toiletries detergent [5]. Generally, CA is widely used as a flavoring, sequestering, and buffering agent in many sectors, especially in foodstuffs, beverages, pharmaceuticals, nutraceuticals, and cosmetics [6]. Additionally, it is also used as a regular ingredient for cosmetic pH-adjustment and as a metallic ion chelator in antioxidant systems. Fascinatingly, CA is also selected to improve the taste of syrups, solutions, and elixirs in the preparations of pharmacies, especially, in mild astringent preparations [2]. Overall, the utilization of CA in several industries grew rapidly in the nineteenth century owing to its interesting physical-chemical properties and environmentally sounds nature. Thus, due to tuneable mechanical properties, biocompatibility, and functionality with advanced material and charismatic in vitro, in vivo properties pretends to explore the applicability of CA in pharmaceutical and biomedical sectors [7]. Ample literature divulged that the CA (molecular formula: $C_6H_8O_7$) is employed as a preservative, emulsifier, acidulant, flavoring, and buffering agent in pharmaceuticals as well as food. It claimed that CA is broadly employed across plentiful industries particularly in food, nutraceutical, beverage, pharmaceutical, and cosmetic products. In addition to this, for the last two decades, the CA is an efficiently applied to cross-linking of different other materials including ultrafine protein fibers for biomedical applications. Besides the aforementioned numerous merits, CA is an excellent, harmless disinfectant against several viruses. On the other hand, owing to its outstanding metal chelating properties, CA is frequently used to clean industrial sites, including nuclear sites contaminated with radionuclides. Furthermore, CA is the most imperative extracting agent in different industrial, agricultural applications. As well, the huge utilization of CA to decrease microbiological activity and thereby getting better stability of concentrates, ex. orange juice makers, who apply the acid to concentrates supplies to customers in the beverage industry [7]. In human metabolism, the citrate ion is oxidized effortlessly and almost completely. Unaltered excretion into the urine takes less than 1%. Interestingly, CA intravenous administration shortens blood coagulation time, but citrate ion functions as an anticoagulant in vitro [2]..

In a nutshell, CA is gaining much attraction from research scientists for numerous advances in pharmaceutical formulations and biomedical applications. Literature survey reported that in the last two decades researchers focused and made huge advances in pharmaceutical applications using CA. For example, despite gas generating agent, acidifier, etc., CA have been holding a huge share

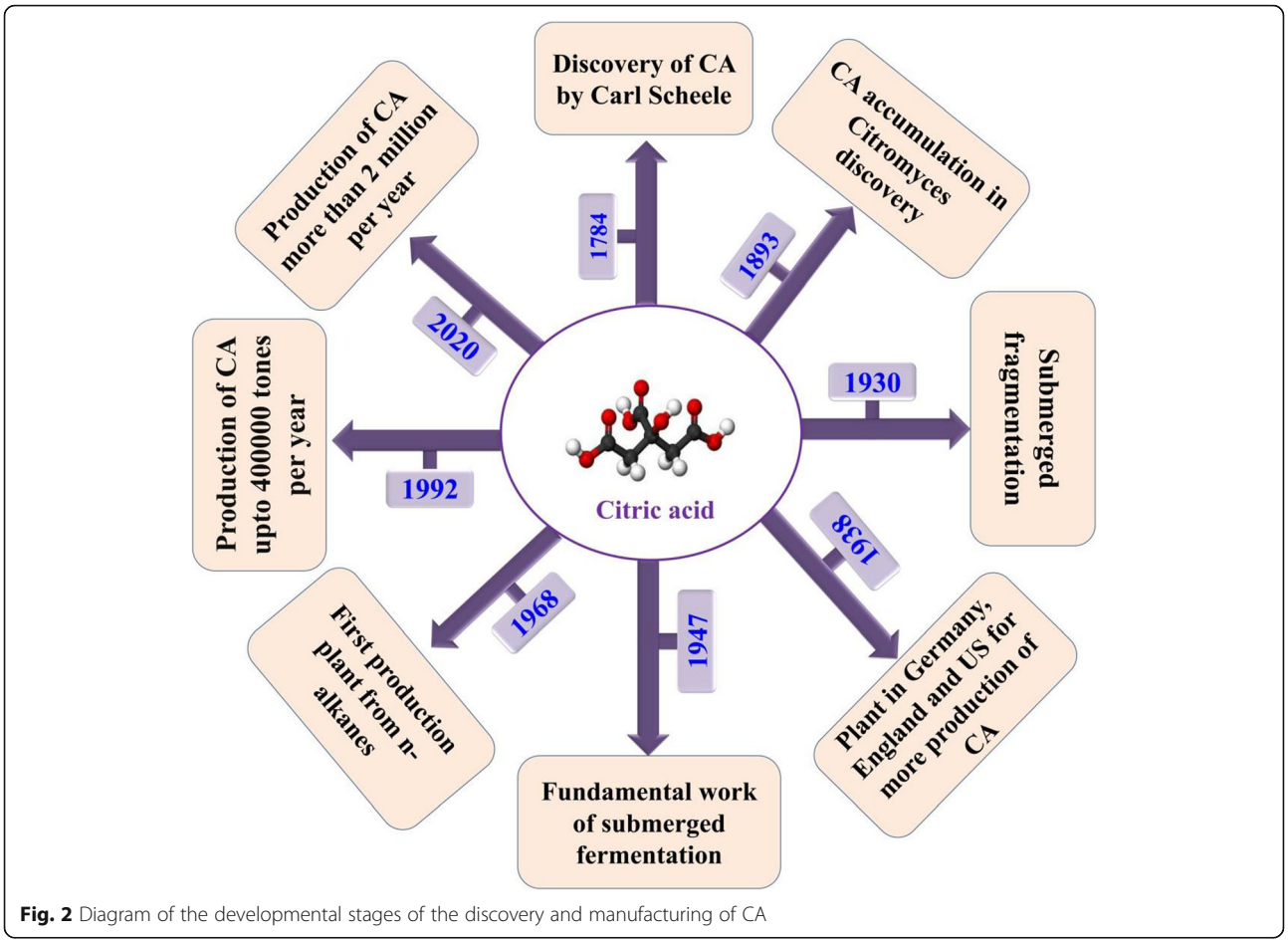

Fig. 2 Diagram of the developmental stages of the discovery and manufacturing of CA

in pharmaceutical applications such as a green crosslinker, crystal stabilizer, capping, and coating agent, novel disintegrant, absorption enhancer, fluorescent material for biosensing, etc. CA is also successfully employed as a dendrimer, monomer, stabilizers, and for the design of novel polymeric (hyperbranched) nanoconjugates.

Concisely, the present article portrays insights into the pharmaceutical applications of CA. In brief, this review article likewise audits the different pharmaceutical applications of CA in designing formulations for effective treatment. Also, it gives a brief review of the ongoing headways in CA as an excipient in pharmaceutical applications, which may assist scientific fraternities to develop advanced formulations and biomedical materials for effective clinical treatment and management.

Main text

Benefits of CA

As per literature, CA is a multifunctional, non-toxic, ready to use, and economical monomer used for pharmaceutical applications [4]. This is a multipurpose natural monomer and playing a crucial role in the control of metabolism, mineralization, neuronal excitations, and renal stone prevention [8]. The pharmaceutical benefits of CA may be because of the presence of three carboxy and one hydroxyl group. CA can be used to contribute prepolymer construction with diol monomers via easy, affordable, and catalyst-free thermal polycondensation that allows the formation of ester bonds and facilitates hydrolysis degradation. The pendant hydroxyl and carboxyl chemistry can be partly preserved, in order to provide inherent versatility for the majority of the substance for the conjugation of bioactive molecules during prepolymer synthesis. In this view, CA containing free pendent chemistry is essential to the production of new pharmaceutical materials with intrinsic adhesives, antioxidants, antimicrobials, fluorescent properties, etc. Due to the availed pendant carboxylate-hydroxylic chemistry, a homogenous network of hydrolyzable ester links/bonds is generated for the cross-linkage to a polymer chain in the process of an additional post-polymerization/polycondensation. Thus, CA is an attention-grabbing monomer, because of its aforesaid characteristics, which has led to the creation of a new paradigm for functional pharmaceutical materials [9]. In addition, CA is an ionic covalent interconnector of

various carbohydrate polymers, which can be destroyed without the release of harmful by-products. It means an aliphatic organic acid with 3 ionizable hydrogen atoms has the additional advantage in biomedical applications [4, 10]. Another benefit of a CA containing pendant groups is that it (carboxylic groups) may be used to graft and combine other macromolecules, as necessary [4]. Notably, CA is one of the organic acids, which is approved by the Food and Drug Administration (FDA) for its pharmaceutical application and considered as generally recognized as a safe (GRAS). It may because of its biodegradability, environmentally friendly nature, ease of metabolization plus elimination, etc. [11, 12]. Therefore, its possible applications goes beyond as compared to the traditional use in food, medicines, and cosmetic products [4].

Pharmaceutical applications of CA

From its beginnings, drug delivery systems are implemented to enhance the effects of the active (drug/therapeutic agent) by regulating their release, targeting location, stabilizing the molecular state, etc. [13]. Recently, various types of pharmaceutical carriers including polymers, nanoparticles, lipid transports, micelles, natural proteins, etc. have gained more coverage. Such carriers have different benefits, mainly in improving the effectiveness and protection of medicinal products. These advanced systems investigated pharmaceutical ingredients and natural active ingredients (hydrophilic and hydrophobic substances) incorporation and consequently its significant delivery. Moreover, targeted/controlled/sustained medicines could be delivered, improved stability, superior bioavailability, etc. For that purpose, assorted naturally obtained revolutionary molecules/excipients are gaining much consideration from research scholars. Similarly, previously reported CA is also attraction-grabbing molecules in the pharmaceutical field. In this subsection, we have discussed the different pharmaceutical and biomedical applications of CA in brief.

CA as a green crosslinker

Literature reported that the straightforward process for inducing chemical or physical connections across polymer chains by supramolecular interactions or covalent bonding is called cross-linking. While many chemical cross-linking agents were used in pharmaceutical applications. But, due to large availability, lowest cost, and particularly non-toxicity, a lot of attention was paid to CA [4]. From its inceptions, assorted crosslinkers viz. epichlorohydrin, glutaraldehyde has been extensively preferred to stabilize PVA-based hydrogels. Unfortunately, such types of crosslinkers are exhibited severe toxic effects and that affects the overall applications in biomedical/pharmaceutical. In this context, the polyvinyl alcohol (PVA), CA, and silver nanoparticles (AgNPs)-based pH-sensitive drug delivery system (PVA-CA-AgNPs) has been established through green, facile, and one-step synthesis routes. In brief, Sabzi et al. developed the PVA-CA-AgNPs nanocomposites using CA as a non-toxic and green crosslinker. Fascinatingly, PVA has no required ionizable functional groups to illustrate pH-sensitive behaviors. In the main, CA is reacted with hydroxyl and amine functionalities of respective biopolymers and accordingly acts as a green crosslinker. In addition to notable properties of CA including stabilizer and crosslinker, it also provides the pH-responsive property and antibacterial activity to PVA hydrogel by the free pendant carboxylic acid of CA. Furthermore, citrate ions in PVA/CA/Ag^{+} solution offer multiple roles such as the development of AgNPs and act as a complex-forming agent, stabilizing, and reducing agent for silver ions. The esterification reaction involving the CA containing carboxyl groups and hydroxyl groups of PVA leads to the cross-linking of PVA (Fig. 3). Based on that PVA/CA/AgNPs nanocomposite containing final hydrogel accomplished the desired aim through solution mixing method using water (green solvent) followed by an annealing activity. Finally, the release of ciprofloxacin (CIFLOX) from hydrogels was found to be more sustained when AgNPs assimilated in the CIFLOX-loaded hydrogel. Additionally, the combination of CA, AgNPs, and CIFLOX into the PVA matrix supplies a powerful antibacterial activity against *Staphylococcus aureus* (*S. aureus*). Overall, the conjugation of CIFLOX and pH-responsiveness nanocomposite hydrogels offers a green and low-cost fabrication route. Therefore, CA (green crosslinker) centered hydrogel can be a perfect promising material for colon-specific oral drug delivery and wound dressing approach [14].

Carboxylic acids like CA have appeared to effective and advanced properties for the products developed from both carbohydrates and proteins. In reality, CA has been recommended to be an excellent biocompatible crosslinker that can come up with desired properties. It enhances the adhesion and production of the cells. In 2020, electrospun PVA fibers have been engineered and later cross-linked using CA (Fig. 4). As per the formula of CA, each CA group comprises 3 COOH groups. The esterification reaction among CA and PVA resulted in crosslinking network and finally increased the molecular weight of PVA (PVA-CA conjugates). In this study, the sum of carboxylic groups for cross-linking with hydroxylic PVA groups also rises as the percent of CA rises that resulted in increased crosslinking levels. In brief, electrospun fibers were examined for their tensile strength, thermal stability, water stability, elongation, morphology, and biocompatibility. Owing to the presence of the

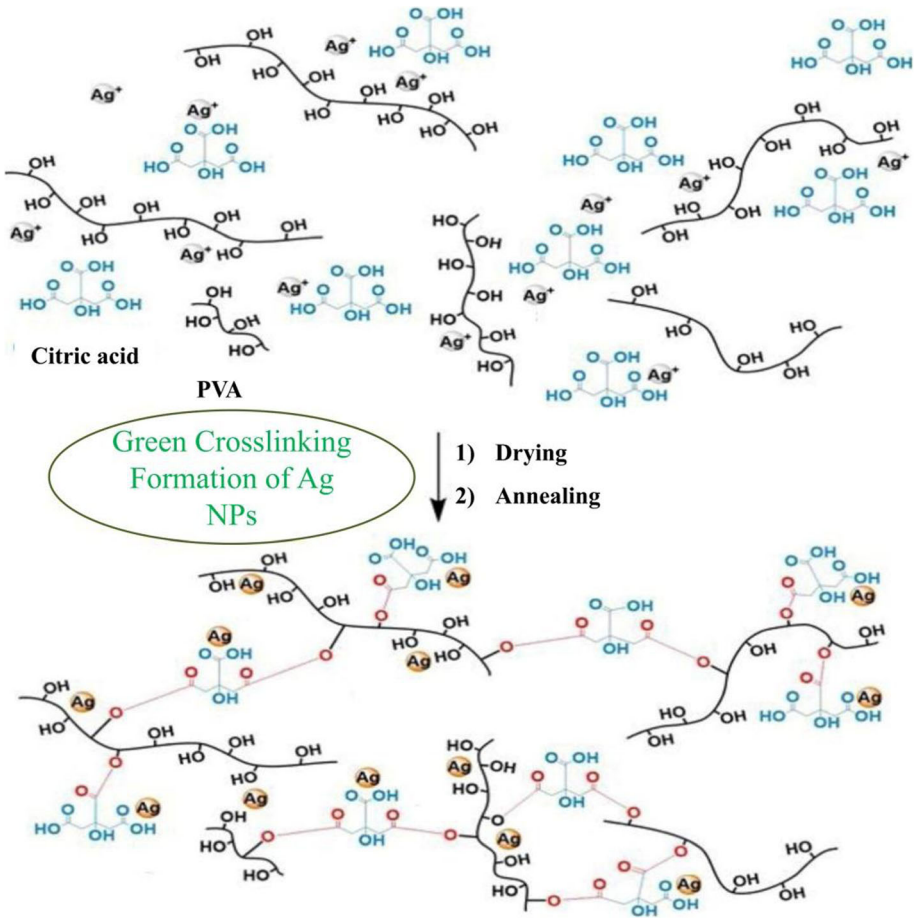

Fig. 3 Diagrammatic representation of simultaneous PVA cross-linking with CA and AgNPs formation. ["Reprinted (adapted) with permission from [14]. Copyright 2019 Elsevier."]

COOH group in CA offers the crosslinking with PVA and finally the stability to nanofibers. Highly hydrophilic PVA has been produced into water-stable electrospun nanofibers via utilizing CA (green crosslinker). In this pioneered study, the potential of CA to crosslink electrospun PVA nanofibers provides good mechanical properties, thermal stability, and morphology. Interestingly, the film's tensile strength gradually increased with increasing CA concentration. On the whole, the aqueous stability plus biocompatibility of the abovementioned electrospun nanofibers offers a way to design suitable material for tissue engineering plus other medical applications [15].

Sidra et al. developed iron oxide (Fe_2O_3) magnetic nanoparticles (MNPs) and functionalized by CA (Fe_2O_3-CA-MNPs). Wherein, CA binds with the magnetic surface via carboxylate, and this helps to convert MNPs into hydrophilic nature which is the major need of biomedical materials. For bioactivity of MNPs, they procured superparamagnetic behaviors with high saturation magnetization. For this motive, they have reported an application centered sol-gel route for the fabrication of Fe_2O_3 nanoparticles (NPs). In this study, the CA has been adsorbed on the surface of MNPs along with at least one free group (C-O or C=O) of carboxyl which converts it into hydrophilic nature and prevents the agglomeration which allows the conjugation with the specific drug/active agent. Furthermore, the zeta-potential investigation also established the functionalization of MNPs with CA and less accumulation of MNPs than un-functionalized MNPs. Also, it showed a zone of inhibition of ~ 36 mm against *bacillus* bacterial stain. The presence of CA increased the antibacterial activity which may due to the rise in reactive oxygen species. Therefore, the CA (0.3 M) functionalized MNPs (superparamagnetic) could be a successful candidate for several applications such as magnetic cell labeling, targeted drug delivery, cancer treatment, magnetic hyperthermia, etc. [16]. Ghorpade et al. reported β-cyclodextrin (β-CD) grafted hydroxyethylcellulose (β-CD-HEC-CA) hydrogel films for the precisely controlled release of ketoconazole, using CA as a crosslinking agent. The presence of free COOH and OH groups in the CA increases the loading

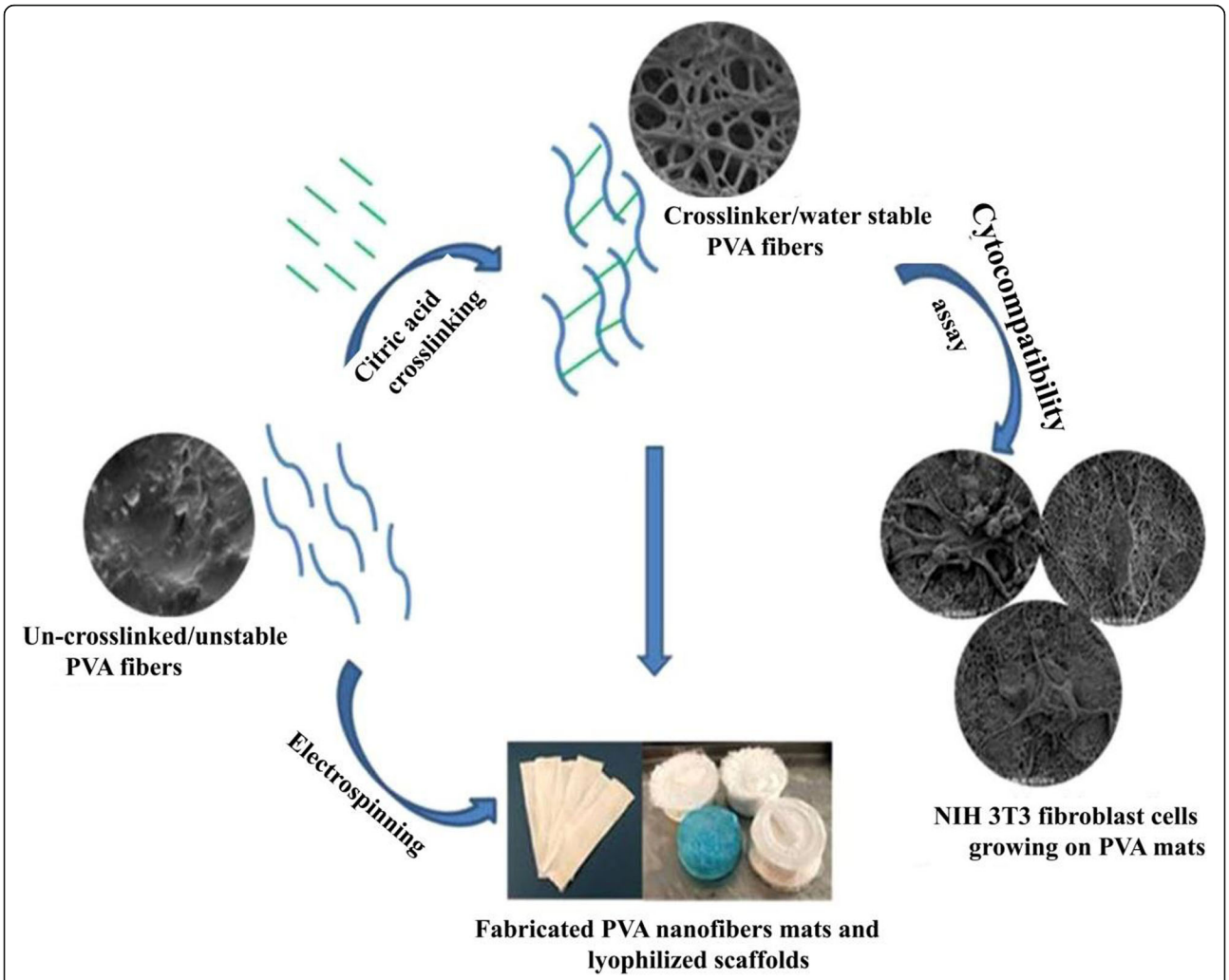

Fig. 4 Representation of stable crosslinking electrospun PVA nanofibers with CA for medical applications. ["Reprinted (adapted) with permission from [15] Copyright 2019 Elsevier."]

of poorly soluble weak bases by ionic interactions and hydrogen bonding. CA convened biocompatibility as well as interpolymer cross-linking in the hydrogels. It also stabilizes the drug-β-CD complex. Free, non-reacted COOH of CA can be deprotonated in an aqueous system, whereas ketoconazole is protonated in an aqueous system. This contributes to an electrostatic attraction between the hydrogel containing COO_2 groups and the ketoconazole, which increases drug loading. Interestingly, the release rate of ketoconazole increased due to the increase in drug loading and reduction in crosslinking. Overall, such a type of combination can be used for extended-release of active up to 12 h [17]. In 2020, Hussain and co-author developed the cross-linking of a glucuronoxylan obtained from *Mimosa pudica* (MP) seed. It offers stimuli (biomimetic) approachable properties, ex. pH, saline, and solvent responsive swelling and deswelling. Furthermore, cross-linked MP hydrogel (CL-MPH) has been formulated using the esterification method of MPH using CA as a cross-linking agent. In brief, different products of a cross-linking degree were prepared with diverse CA concentrations and identified through FTIR spectroscopic analysis. CL-MPH was synthesized by MPH with CA, wherein *N*,*N*-dimethylacetamide has been used as a reaction medium. In this study, the CA transforms one side into anhydride by heating which is an extremely reactive species contributing to an ester by means of MPH hydroxyls (e.g., glucuronoxylan). The secondary terminal of CA is also transformed to anhydride under continuous heating and then connected with an alternative glucuronoxylan chain (Fig. 5). CL-MPH (with 5% CA) shows elevated swelling at pH 6.8, 7.4, and in deionized water, whereas minor swelling was found in pH 1.2 media. The swelling followed the kinetics of 2nd order as predicted for highly porous materials, while release kinetics of diclofenac was found to zero-

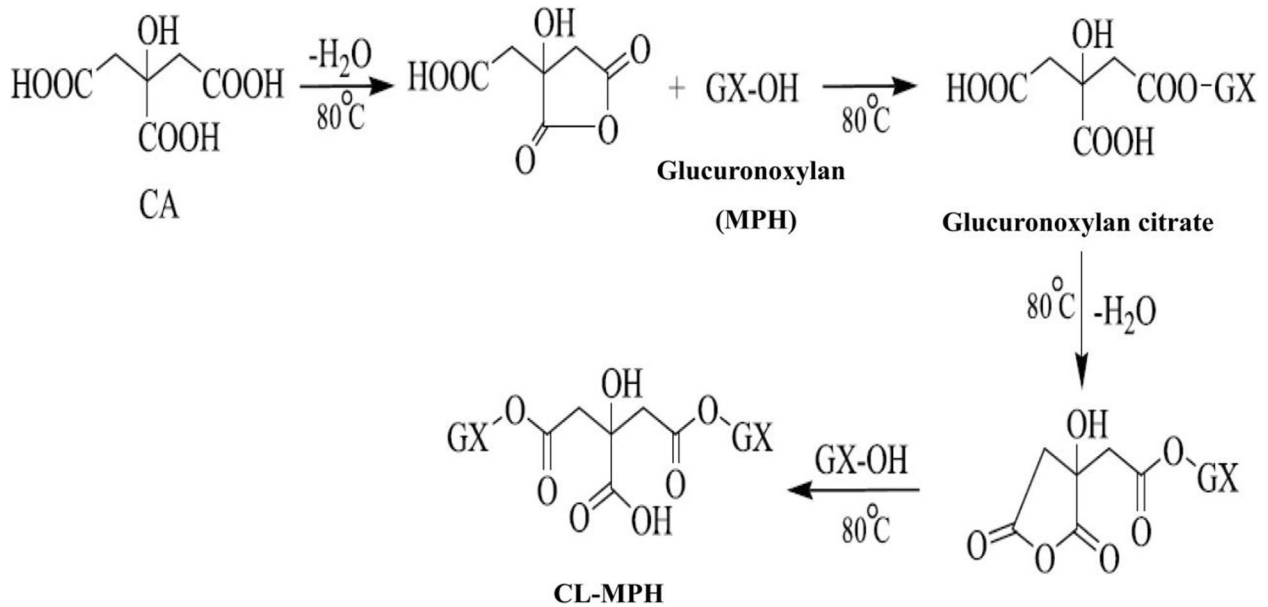

Fig. 5 Synthesis scheme for CL-MPH using CA and MPH. ["Reprinted (adapted) with permission from [18]. Copyright 2019 Elsevier."]

order that suggesting the perfect continuous releasing existence of CL-MPH. In the future, cross-linked glucuronoxylan can be designed using CA to keep its swelling response intact for various applications viz. development of stomach-safe oral formulations which can preferentially be used for ulcer patients [18].

Literature reported that the CA is inexpensive, safe, non-toxic, and naturally available. Poly-carboxylic acid could cross-link the proteins by the development of novel intra/intermolecular amide linkages through a nucleophilic substitution reaction. In a nutshell, CA is an innovative gelling agent. In a few studies, it productively engaged to alter the resulting protein-stabilized emulsions into stable gels. Mohammadian et al. addressed the production and evaluation of gels from emulsions that were stabilized by heat-denatured, nano fibrillated whey protein isolate (WPI) via CA and $CaCl_2$ as cold-gelling agents. The formulation of emulsion gels by CA-mediated cross-linking has been constant than those formed through $CaCl_2$. In this relation, the formation of amide bonding has been identified as the key mechanism for the linkage of CA-mediated food protein (CA-WPI) by CA containing carboxyl groups and protein-containing amino groups. This hydrogel displayed a high aptitude to hold water plus retain oil. Hence, the gelified emulsions using CA are an alternative as compared with $CaCl_2$-induced gelation [19]. Demitri and co-workers reported that hydrogel using sodium carboxy methylcellulose (Na-CMC), hydroxyl propyl methylcellulose (HPMC), and CA (crosslinker) [20]. Hashemi and co-workers developed whey protein hydrogel loaded with nanostructured lipid carriers (NLC) and gelled with help of CA (crosslinking agent). In this study, CA (crosslinking agent) was successfully implemented for cold-set gelation of heat-denatured whey proteins. Interestingly, CA gelation of whey proteins rather than the conventional $CaCl_2$-induced gelation resulted in much firmer gels with higher WHC (water holding capacity) and lower syneresis. In this study, the CA-based gelation resulted in the development of more hydrogen bonding (N-H) in the gel. Then, the new amide bonds among CA containing carbonyl and amino groups (deprotonated) whey proteins confirmed the disordered polypeptide chains. The higher firmness of CA-based gels was attributed to finely meshed gel microstructure. Synergism was observed between nanostructured lipid carriers (NLC) loading into WPI-CA gel and CA gelation instead of $CaCl_2$ gelation. CA crosslinking made whey proteins more hydrophilic. Hence, CA can be used as a crosslinking agent, for NLC loading [21]. Farjami et al. reported that the formation of the bulk hydrogel of the whey protein cross-linked microgels (WPI-CA) using CA. The CA was used for pH adjustment and cross-linking of whey proteins before or after their microcalcification by heat. In that, CA as a substitute of hydrochloric acid in the microcalcification method of whey proteins that reduced particles up to 80 nm to 130 nm and 100 nm to 150 nm for post- and pre-microgelification cross-linked samples, respectively. It may due to influence of CA as intra- and intermolecular cross-linker. Notably, the extended CA cross-linking of whey proteins before the process of microgelification resulted in an extensive cross-linking of protein units followed by retained proteins α-helical structure. The bulk hydrogel formation of

pre-cross-linked microgels shows a dense microstructure. As highest firmness and water-holding capabilities associated with those made of predictable and post-cross-linked microgels. It solely relied on the number of carboxy groups present in pre-cross-linked microgels. Furthermore, owing to the covalent cross-linkages development in the structure of whey protein microgels by a non-toxic reagent, the bulk hydrogels along with altered mechanical and structural properties can be fabricated [22]. The chemical crosslinking between Na-CMC and HPMC has been reported by Dharmalingam and Anandalakshmi. In this study, after condensation reaction, while heating, CA generates cyclic anhydride and then cyclic anhydride-cellulosic hydroxyl groups formed the ester bonding. After this step, the next cyclic anhydride was formed due to further heating that contributes to esterification with the cellulose-containing hydroxyl group. Interestingly, in the present formulation, the ester band intensity at 1720 cm^{-1} to 1735 cm^{-1} increased with a rise in CA. In this study, it was found that the crystallinity, swelling degree, and water contact angle of designed hydrogel films depend on NaCMC-HPMC (2 wt%). They reported that the crystallinity, swelling degree, and water contact angle of these films were decreased with a rise in CA from 5 to 20% (by weight). The designed hydrogel films' tensile strength was decreased while elongation at break (percent) increases with CA concentration, swelling ratio, the contact angle of water increased. In addition, the methylene blue (MB) loading capacity was found to be more as compared to the tetracycline. These hydrogel films showed significant antibacterial activity after 3 days of release at 37 °C in phosphate buffer saline (PBS, pH 7.4). These results powerfully mention that the ready formulation of hydrogel films can be selected as potential wound healing constituents. Hence, hydrogel films using CA can be preferred as a probable wound curing ingredient [11]. The biopolymers-based hydrogels result in various applications in several fields viz. packaging materials, biosensors, drug delivery systems, and agricultural practices. Interestingly, nowadays CA has been widely used as a crosslinking agent in starch. In 2019, Bruno and co-author have developed starch/xanthan hydrogels through expulsion and 24 thermo-pressing processes using sodium hypophosphite (SHP) as the catalyst and CA as a crosslinking agent. They were prepared hydrogels using different concentrations of CA (example: 0.00, 0.75, 1.50, and 2.25 g/100 g polymer). Herein, the heating of CA resulted in an anhydride and then interact with xanthan gum containing –OH. Finally, it resulted in the grafting of the citrate group in xanthan gum. Further, this grafted functional group can interact with the –OH group of another molecule and resulted in crosslinking. In that, the xanthan gum containing –COOH group and CA containing –OH groups resulted in an esterification reaction. CA offers less strength than non-crosslinked hydrogels, which may be coupled to acid hydrolysis of the polymer chains. Also, CA showed improvement in the storage of the hydrogel. The results of CA in the mechanical properties, particularly the rise in elongation, are helped by the hydrolytic action that can be beneficial for numerous applications of hydrogels. To further study, the properties and functionalities of the hydrogels formulated should be confirmed [23]. In another study, Rocha-García et al. developed poly (ethylene glycol) diamine (PEG-D)-based hydrogels using CA as crosslinking agents, glutaraldehyde (GTA), and gelatine (GEL) as hydrogel vehicle (Fig. 6). Herein, the PEG-D containing NH^{3+} (protonated at pH 5) and CA containing COO^{-} (deprotonated) physical weak interaction resulted into the low strength material (Fig. 6). The lower stiffness on the bonds which are generated via intermolecular forces like NH^{3+} and COO^{-} containing attractive forces and the GEL and CA containing non-ionized carboxylic acid functional groups resulted in hydrogen bonds that provide the high flexibility for hydrogel containing 3D network (Fig. 6). They showed the new substitute by using CA as a crosslinking agent to produce new low-cost 3D polymeric scaffolds. Herein, CA offers renewable resource-based substances, mainly for industrial uses by the fermentation of carbohydrates, starch, or glucose. Besides, CA has a polyfunctional non-toxic nature that utilizes oxygen as part of cellular respiration. Furthermore, CA has high-quality properties viz. solubility, high cytocompatibility, and obtainable at the lowest cost. This developed hydrogel offers the anomalous drug release mechanism for tramadol. Mostly, owing to the high range of PEGD and GEL, the biocompatibility and biodegradability with the supplementary advantages of CA capacity provides noticeable cytocompatibility. Overall, the PEGD:CA/GEL hydrogels are suited for an extensive array of in vivo biomedical applications [23].

Kanaf and co-worker's (2019) developed cross-linking of highly porous CMC/poly (ethylene oxide) (PEO) composite (PMC) hydrogel films for controlled release applications using CA. In that, the CMC plus PEO has been selected as the chief materials for the designing of hydrogel formulation. It may due to their excellent properties (such as non-toxic and biocompatibility) for drug delivery applications. The non-toxic chemical CA has been preferred as a cross-linker. Herein, the CMC contains the carboxymethyl and hydroxyl functionalities, whereas the PEO contains only hydroxyl functionalities. In the case of CA, the carboxylic acid groups majorly crosslink with CMC containing a hydroxyl group. Besides this, lower crosslinking with PEO has resulted that due to the presence of only hydroxyl groups. The CMC/PEO hydrogel swelling behavior remained assessed based

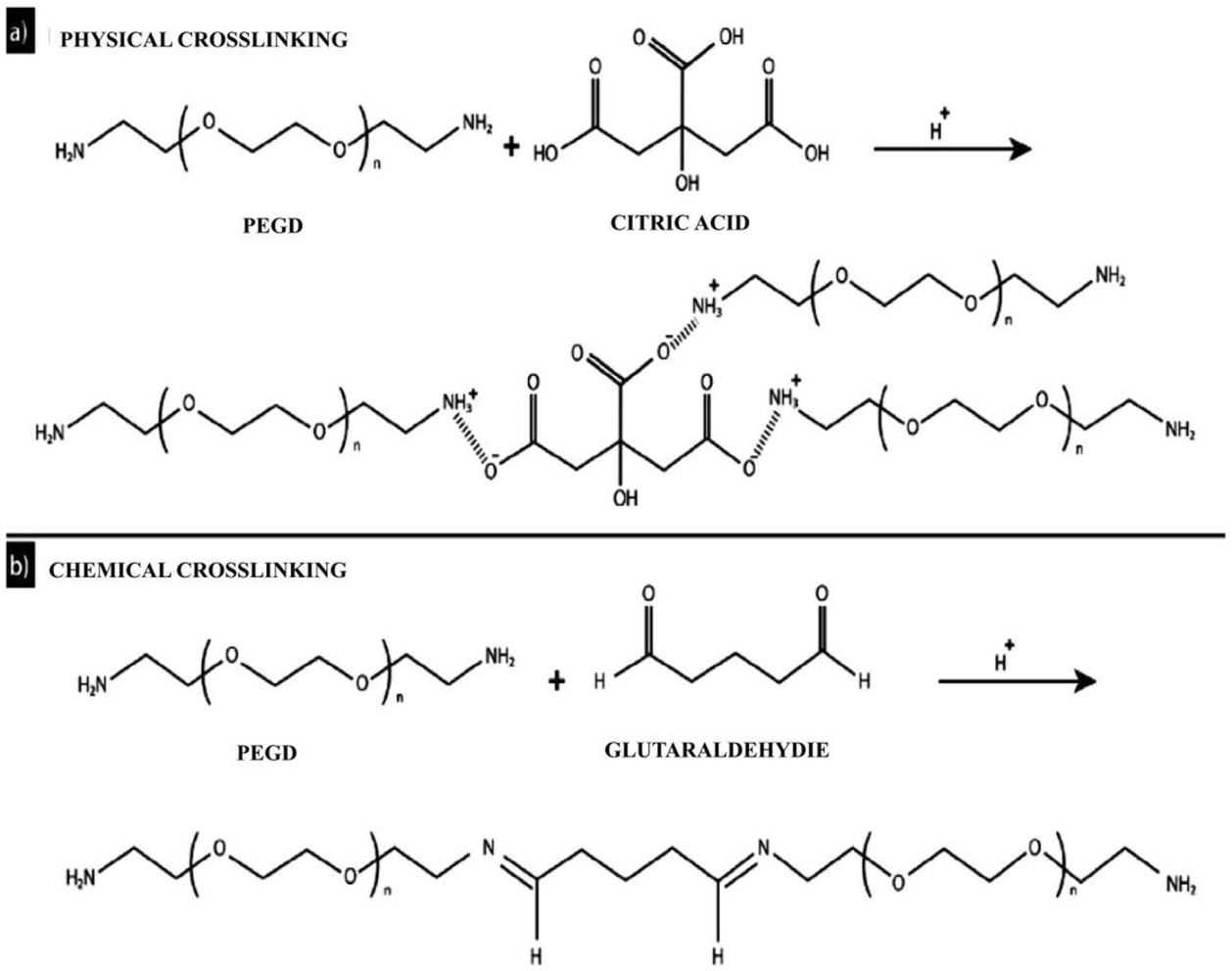

Fig. 6 Mechanism involved in PEGD: CA/GEL hydrogels. **a** Physic crosslinking with CA. **b** chemical crosslinking with GEL. ["Reprinted (adapted) with permission from [23]. Copyright 2017 Elsevier."]

on four limits; a ratio of CMC to PEO, a percentage of CA, temperature, and duration of curing. The MB released from hydrogel was found to be pH-dependent. In brief, MB was released with low concentration in the acidic environment contrary to the basic environment. Furthermore, a maximum swelling ratio has been obtained when the CA concentration reached 10% w/w. It means that the CMC/PEO hydrogel swelling ratio has been increased with the percentage of CA increased (up to 1% w/w). It decreased afterward by more than 10% w/w CA. The progressive proportion release of CMC/PEO hydrogel for MB as a controlled drug distribution has been reached below 25% for the acidic and basic environment even after 48 h. The outcomes of this investigation expressed that the CMC/PEO hydrogel has a towering potential to be preferred in controlled drug release for biomedical applications [24]. Ghorpade and coworkers reported that the CMC-PEG hydrogel films used a non-toxic cross-linking agent (CA). It provides the controlled release of ketoconazole (a hydrophobic drug). Briefly, CA forms cyclic anhydride, which esterifies the responsive OH groups and together polymer chains. The PEG containing terminal OH groups exhibited as a most reactive and surely contributed to the esterification reaction with CA. Then, it leads to the CMC-PEG hydrogels formation. Generally, CA forms cyclic anhydride at high temperatures (above 60 °C), which esterifies the neighboring polymer chains' containing reactive OH. It leads to the formation of ester crosslinking. Furthermore, the CMC containing C6-OH group (OH attached to C6–anhydroglucose unit) and PEG terminal containing OH are main reactive groups and can be readily involved in a CA-esterification reaction for leading to the production of CMC-PEG. Moreover, the CMC degree of substitution was found to be 0.7. Therefore, there is an opportunity for the participation of C_2-OH and C_3-OH of CMC in the esterification reaction. The increase in the concentration of CA above

20% shows the extensive crosslinking that resulted in tough films along with slight swellability, which increases the curing temperature plus therapeutic time [17]. CA-derived biomaterials are novel materials that contribute functionality to the ester bond formation/crosslink, balancing the polymer network hydrophilicity, improving hemocompatibility, supplying hydrogen bonding, and offering additional binding sites for further bioconjugation functionality. Mali et al. developed the carboxymethyl tamarind gum (CMTG) hydrogel films (CMTG-CA) using CA as a crosslinking agent for the delivery of moxifloxacin hydrochloride. In that, crosslink formation between CA and CMTG is a response of esterification reaction. In the case of CA heated at a higher temperature, there are major changes in the development of intermediate cyclic anhydride, which is accountable for the expansion of crosslinking with CMTG. Further, the designed intermediate cyclic anhydride can open under the action of polysaccharides containing OH functional groups with esterification. It is important to a new carboxylic acid unit into a carboxylic acid, which has the ability of structured a new intramolecular anhydride moiety along with the neighboring carboxylic acid unit. Herein, the arrangement of ester linkage among CA and CMTG was established and confirmed by attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR). It confirmed the role of CA as a novel cross-linking agent for CMTG. The overall study concludes that the studies on the CMTG hydrogels can be a variety of prospective applications in the agricultural, biomedical, and pharmaceutical fields [25]. As we know, CA has been employed in polysaccharide chemistry, for decreasing the water sensitivity of packaging materials, etc. CA can also be preferred based on water-soluble cellulose by-products. CA is also employed for finding superabsorbent hydrogels from CMC and HPMC, and for improving the thermal stability, tensile strength, and decreasing the overall dissolution of starch films into provided water and formic acid. Ample literature claimed that CA is used for the preparation of in situ cross-linkable biodegradable polymers for cell delivery. Uliniuc et al. developed the improved starch-based hydrogels (starch-CA), which cross-linked through CA for drug distribution systems for levofloxacin. In brief, CA-derived biomaterials containing CA provides precious pendant functionality that involved in ester bond cross-linking arrangement, enhancing hemocompatibility, providing hydrogen bonding, balancing polymer network hydrophilicity, and provides added binding sites for aimed bioconjugation to confer additional functionality. Furthermore, the effect of numerous amounts of CA and cross-linking time used not only experimental program but also the changes in the morphology and/or stability of the hydrogels in water. They reported that increasing CA concentrating and cross-linking time lead to a higher density of the finally obtained network, and consequently to a decreased water/drug retention. Besides that, in the case of hydrophobic hydrogels, the reduced level of hydration of the matrix resulted in a long-time release of levofloxacin. Therefore, in the upcoming future, to assess the ability of these hydrogels to acts as biomaterials, further studies including cell compatibility need to be conducted [26]. Shafagh and co-workers reported that the pH-sensitive and antibacterial gelatine CA/Ag nanocomposite (CA-Ag-Gelatine) hydrogels using a solution mixing method with the presence of CA (green crosslinker). In this study, the gelatine (natural biopolymer) has been crosslinked with CA in the existence of AgNPs gelatine-based hydrogels. Due to the multi-carboxylic structure of CA, the chemical reaction could take place among the carboxyl groups on CA and the amine groups on gelatine. Besides this, a composite combining CA-cross-linked gelatine hydrogel with AgNPs inclusions with probable for biomedical applications was successfully reported in this work. Hence, the adding of CA into cross-linked-gelatine/Ag nanocomposite hydrogels shows the pH (7.4) dependent swelling and drug (cefixime) release. Overall, this developed hydrogel can be used for stimuli-responsive drug transport applications [27].

Overall, due to the noticeable properties of CA (green crosslinker), it is playing a crucial element in the development of drug-loaded formulations such as a hydrogel, films, etc. for targeted/controlled release of diverse types of drugs/actives. Therefore, in the future, CA can be a better substitute for chemically synthesized crosslinkers in pharmaceutical and biomedical applications. Despite these notable advantages, a more scientific explanation is required for the influence of physicochemical and mechanical characteristics in CA cross-linking.

CA as a co-crystal

Effervescent products are variously used in pharmaceutical, nutraceutical, food, agriculture, detergent, and cleaning sectors but the major problem of the chemical and physical instability of the effervescent products during production and storage. In 2020, Paigre and co-workers reported that the co-crystallization process of CA and sodium bicarbonate (SBC) is an important component of effervescent products. CA is hygroscopic and leads in the case of a diminutive amount of moisture inducing product volatility to an uncontrollably autocatalyzed reaction in the chain. The acid amide dimer binding and co-crystal-layered structure of CA nicotinamide limits the contact between moisture and CA creates non-hygroscopic moisture and improves product stability. This study resulted in a computational investigation into moisture interactions with different crystal

surfaces. In this study, nicotinamide (NIC) was preferred as a co-former molecule as widely accepted in the formulations of nutraceutical supplements. They demonstrated an application of co-crystallization to tailor the hygroscopic nature of the CA by blocks the water interaction. CA has shown its potential in the manufacturing of stabilized effervescent products, which will significantly lower acidic agents, ex. the CA, maleic acid, tartaric acid, malic acid. The sorption constant shows well the strength of the interaction of water vapor with the crystal surface and calculated with GAB and Y&N equation. They concluded that sorption CA is higher than CA-NIC co-crystal. Batch with the acidic component in the form of co-crystal, i.e., CA-NIC co-crystal, shows good stability as compared to the CA as the acidic component of the effervescent product [28]. Overall, in the future, NIC can be used as an ideal co-former for CA. There is a need to study the effect of co-crystallization on the stability of effervescent products.

CA as a pH modifier

In 2019, Luming Li and colleagues divulged the systematic 2D hexagonal mesoporous silica (SBA-15) progress under medium poly-carboxylic acid (e.g., CA or oxalic acid) without establishing mineral acid and inorganic salt. Carboxy groups (COOH) within molecules of organic acid playing an imperative job in the high-end mesoporous silica materials synthesis, to their bridging effects on both non-ionic compounds and silica precursors. The more organic acid is a carboxylate, the less organic acid has been taken. For high-quality SBA-15-CTA (CTA: the material prepared under CA medium) materials development, the accumulation of CA was less than 0.0010 mol/L (pH > 3). The microstructuring and rod-like particle size can be engineered by different amounts of acid type and their preparation conditions. The synthetic parameters viz. acid types, reaction temperature, concentration, and aging time have been systematically examined. Notably, organic acid with additional two COOH groups based products. The acid possesses more COOH groups' which offers less dosage requirement. The low concentration of CA 0.0010 mol/L has been used for SBA-15-CTA mesoporous materials fabrication. Worthy to mention that the large molecular acid along with poly-carboxylic groups can provide a better bridge between non-ionic copolymer micelles and silica precursors. Thus, it assists in the construction of rod-like mesoporous silica materials. Besides, the synthesis approaches under weak organic acid medium present a distinctive industrialization view, considering their low acidic index [29].

Nath and co-authors have reported sterculia gum preparations in the type of an osmotic core tablet (AZA@CA) designed for colon-specific azathioprine (AZA) distribution using CA as a pH modifier. Briefly, CA has been used as a pH-regulating excipient in the advanced formulations. They were expected to reduce the pH of the developed colon-specific core tablets to a suitable therapeutic level. In this study, the incorporation of CA also helps to understand the mechanistic behind the absorption and expansion by sterculia. Furthermore, the concentrating of CA increased the AZA release in colonic pH. It may due to the rapid solubility of CA by colonic fluid. In addition, the inclusion of CA increased the basic amino group's ionization in AZA and further increased its release (85%) in dissolution media (7.4 pH) in 12 h. Hence, the application of CA as a PH modifier can be a better substitute for colon-specific (targeted) drug delivery systems [30].

CA as a fluorescent material for biomedical applications

CA has various multifunctional applications in different areas such as drug delivery, biomedical labeling, in vitro/in vivo bioimaging, and sensing in cellular/environmental samples. The CA attracted significant attention as a revolutionary precursor to formulating fluorescent materials due to its high quantum yield (QY). In 2019, Liu et al. developed a highly photoluminescent molecule with the help of CA and cysteamine through hydrothermal synthesis (Fig. 7) In this study, CA and cysteamine were selected to manufacture an extremely high luminescent molecule (i.e., 5-oxo-3,5-dihydro-2H-thiazolo [3, 2-a] pyridine-7-carboxylic, TPCA) using a one-pot hydrothermal reaction followed by a simple purification process. Herein, application of high temperature plus high pressure during synthesis of TPCA, the reactant materials (CA and cysteamine) continuously suffers the dehydration and finally converted into TPCA. Interestingly, Au^{3+} selectively quenched the strong fluorescence of used TPCA in an aqueous solution with a 51 nM detection limit. Interestingly, CA has obtained broad interest because it can consider as a low-cost precursor to produce emerging highly fluorescent materials viz. carbon dots (CDs), biodegradable photoluminescent polymers, small molecular fluorophores (SMFs), etc. Further, CA-based CDs (CA-CDs) furnish several merits including outstanding biocompatibility, modifiable emission wavelengths (from blue to red), high photobleaching resistance, and simple synthetic routes. The fluorescence QY of TPCA was 87.1% by using quinine sulfate (reference). Briefly, the hydrothermal route provides highly luminescent molecules, i.e., TPCA crystals from CA and cysteamine with the help of simple purification procedures. This effectual strategy will expand the purpose of CA-based SMFs and it can be supportive of the invention of other environmental sensors [30].

Targeted tumor imaging and successful in vivo gene delivery are some of the foremost obstacles in the

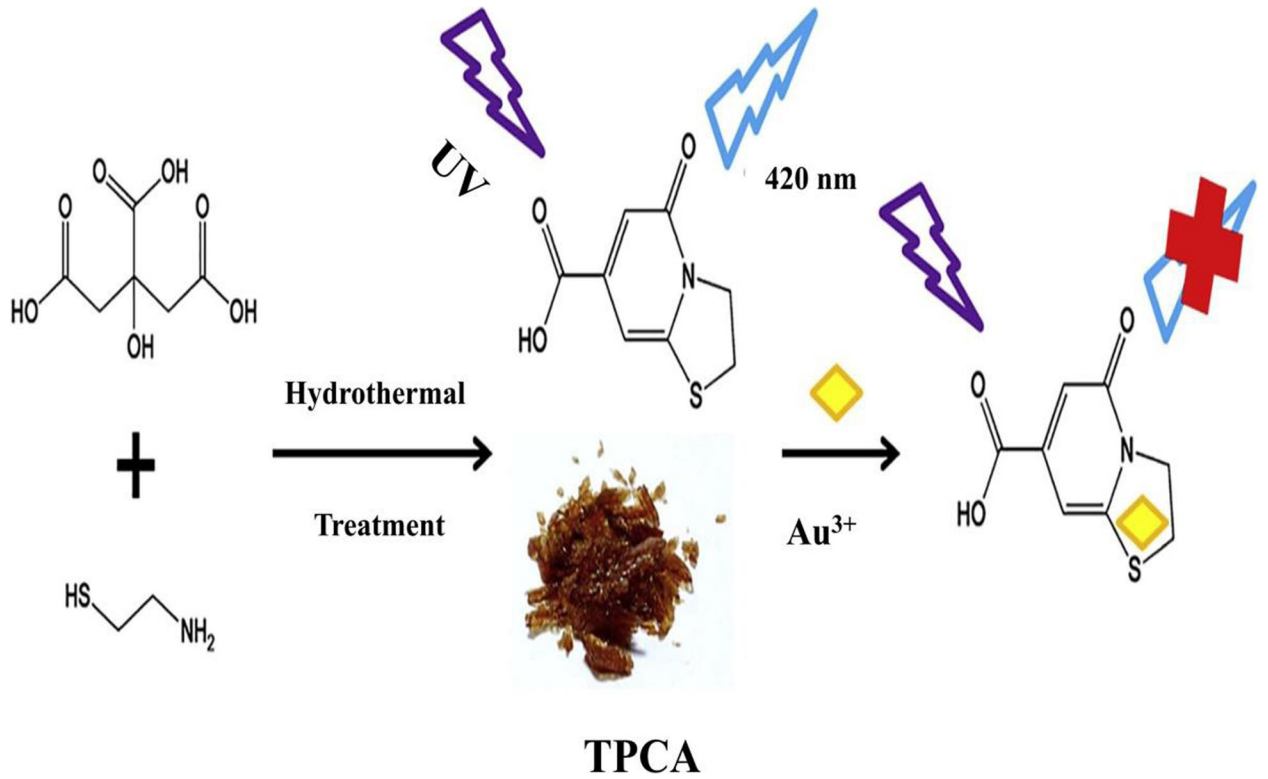

Fig. 7 Schematic representation of TPCA synthesis using CA and cysteamine through hydrothermal route for detection of Au^{3+} ["Reprinted (adapted) with permission from [30]. Copyright 2019 Elsevier."]

diagnosis and treatment of hereditary cancer. Briefly, the CA-based nano-vectors for intrinsically targeted tumor imaging plus delivery of specific siRNA gene (in vitro or in vivo) developed by Wang and co-authors. The developed CA-based polymer offers the intrinsic photoluminescence plus gene loading capacity for achieving specific siRNA delivery plus tumor imaging (in vitro and in vivo). The multipurpose platform was designed from the self-assembling of poly (CA)-polyamine conjugated with folic acid and rhodamine B (PPFR). Finally, PPFR exhibited stable photoluminescent aptitude and successfully bind plus protect the siRNA against enzyme degradation namely RNase. In addition, PPFR also showed superior blood compatibility plus cell compatibility against C_2C_{12}. In comparison with commercial transfection agents, i.e. lipofectamine™ 2000, PPFR provides several advantages viz. equivalent transfection efficiency, higher cellular uptake, efficiently downregulated intracellular p65-expression in A549 cancer cells, etc. Importantly, PPFR efficiently accumulated and labeled the tumor tissue confirmed through fluorescent imaging. In addition, selectively siRNA transport into tumor tissue (in vivo) depends on the tumor containing a nude mice model. CA-based polymers show good biocompatibility, controlled biodegradation, and biomimetic elastomeric behavior. Hence, it offers the biocompatible CA-based multifunctional non-viral vector with intrinsically stable photoluminescence plus gene loading capacity. It initiated the as-synthesized nano-vectors showed intrinsically targeted tumor imaging and intracellular siRNA delivery and gene silencing. This technique used to track live cells demonstrated targeted in vivo tumor imaging and siRNA delivery. Besides, it is recommended that multifunctional CA-based nano-vectors were maybe pioneering biocompatible biomaterials for distinguishing targeted tumor imaging plus simultaneously cancer therapy. Concisely, this work furnishes a simplistic approach to engineered multifunctional biocompatible biomaterials intended for effective and targeted tumor imaging plus gene therapy [31].

More and co-authors have been synthesized blue luminescence CA-graphene quantum dots (CA-GQDs) through various types of equipment viz. furnace, domestic microwave synthesizer, and scientific microwave synthesizers using CA as a green precursor. In brief, CA has been carbonized utilizing the above-mentioned types of equipment at definite reaction conditions. Incomplete carbonized CA can be confirmed due to the presence of C–H and C-O stretching vibration. After that, complete dehydration of CA resulted in aromatic C=C stretching GQDs. Also, during CA-GQDs synthesis, the H bonding can be decreased drastically. The CA-GQDs contain

COOH groups and OH groups on surface help to sensing and loading applications. Fascinatingly, the prepared nanosized GQDs showed blue luminescence at a long wavelength (365 nm). It is supportive for verification of the degree of carbonization of CA used to convert into GQDs. The dispersion heated at 40 °C for 1 h does not show any change in fluorescence intensity while an incremental temperature of 60 °C and 80 °C shows a reduction in fluorescence intensity at a longer wavelength in the UV cabinet. Hence, GQDs from economic sources can be scalable methodology. Besides this, CA can be preferred as a source of carbon material, which can help several biomedical applications [32].

In another study, Kong and co-authors have developed carbon dots (CDs) by hydrothermal method using CA as a carbon source and ethylenediamine (precursor). In brief, obtained CA-CDs presented quasi-spherical morphology with small particle size. Further, doxorubicin (DOX) loading with CDs (CDs-DOX) can be achieved by electrostatic interaction. Finally, the CDs-DOX complexes accomplished a pH-sensitive drug release property. It may due to the DOX's superior solubility into an acidic medium. Interestingly, as pH-sensitive carriers, CDs-DOX complexes can promote the efficiency of drug release in tumors acidic environment. Furthermore, CDs possessed excellent biocompatibility and showed a potential proliferative function in provided L929 cells. Hence, the CDs-DOX drug distribution system demonstrates relatively high loading capacity plus a superior drug release profile (82.0% release in 72 h). Furthermore, the in vitro results confirmed that the CDs-DOX complex offers a higher cellular uptake plus extra effective inhibition capacity on cancer cells in contrary to free DOX. Generally, CDs transfer into the cell via endocytosis as well as passive diffusion and DOX cellular transfer depends on passive diffusion. Therefore, CDs with DOX shows the combination of these mentioned two mechanisms for cellular uptake and ultimately it gives higher cellular uptake that confirms by bright red fluorescence as compared to individual uptake of drug and CDs (Fig. 8). In a conclusion, the nanosized CDs-based DOX may hold great potential for cancer chemotherapeutic applications [33].

CA as a capping agent and coating agent

Ghafelehbashi and co-workers manufactured and characterized the CA; α-Cyclodextrin (α-CD) functionalized Fe_2O_3 NPs (i.e., IONPs) by different approaches. In this study, the CA containing C=O and α-CD containing OH resulted in esterification. Then, the quercetin shows strong interaction with α-CD due to high organic content. Owing to the less binding energy, there is weak interaction among drug molecules and CA. It means the quercetin adsorption on CA/α-CD-IONPs can improve the solubility and chemical activity of quercetin via molecular complexations. Overall, the van der Waals

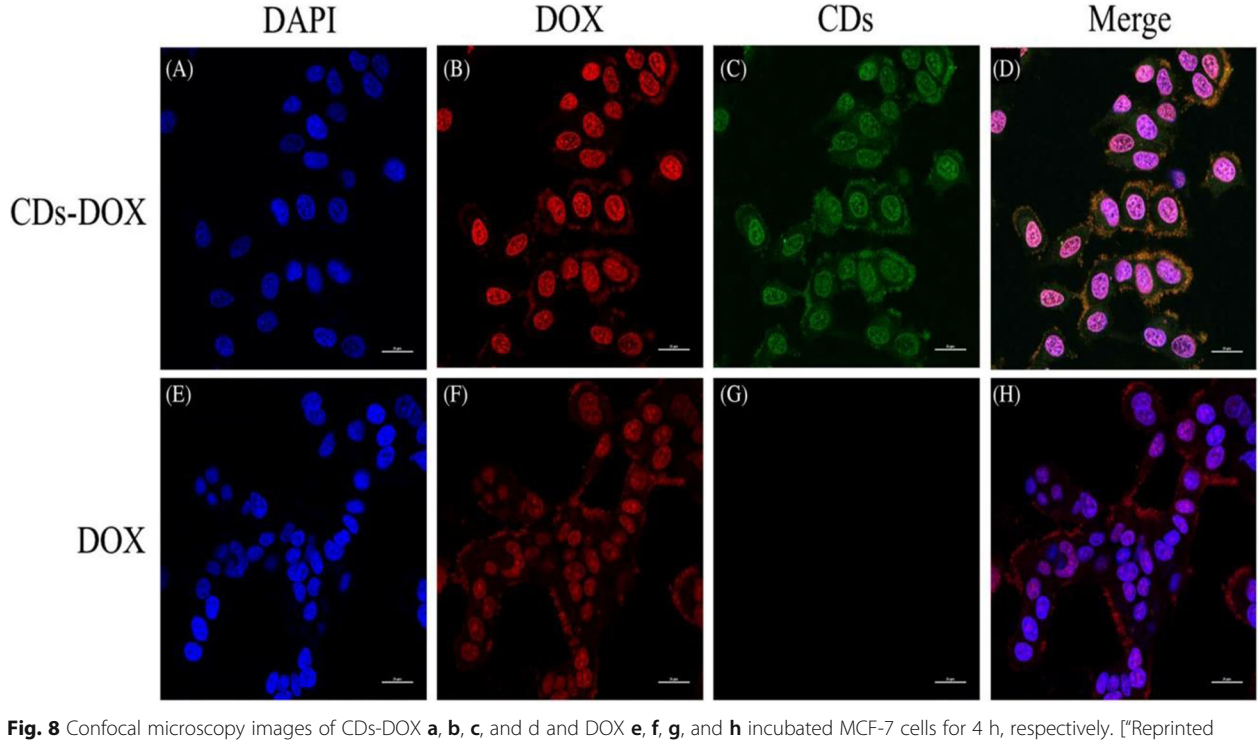

Fig. 8 Confocal microscopy images of CDs-DOX **a**, **b**, **c**, and d and DOX **e**, **f**, **g**, and **h** incubated MCF-7 cells for 4 h, respectively. ["Reprinted (adapted) with permission from [33]. Copyright 2018 John Wiley & Sons Ltd."]

interactions and hydrogen bonding between these molecules are much important to design the CA/α-CD-IONPs. The drug (quercetin) adsorption and release behavior of CA/α-CD functionalized IONPs (CA/α-CD-IONPs) exhibited pH-responsive action. Interestingly, quercetin was loaded into composites at alkaline pH and further released at acidic pH. Besides this, the dry film thickness (DFT) designs were employed to provide an improved understanding of the possible interactions among the quercetin and nanocarrier. The improvement of the IONPs with a composite of α-CD and CA resulted in an even bigger nanoparticle. It may due to the made-up of porous structure in CA/α-CD complex as a result of the strong interaction between CA and α-CD [34]. There is a huge need to functionalize/modify the surface of NPs. It may due to amplifying the shelf life of NPs in the bloodstream followed by passing the MNPs through the cell membrane. Zargar et al. developed the PEG-coated $Zn_{0.3}Fe_{2.7}O_4$ NPs (PEG-$Zn_{0.3}Fe_{2.7}O_4$ NPs) using CA and Fe_2O_3 through hydrothermal reduction reaction. In this experiment, the presence of citrate ions on the NPs surface has assured the long-term stability in water. As the citrate coating on NPs, the surface shows a negative charge that avoids the aggregation of NPs and helps to maintain the stability of NPs. In addition, the cell membrane contains a negative surface charge, so the possibility of NPs taken up by the cell membrane is entirely diminished. The occurrence of the primary coating of citrate avoids an extreme reduction of heat generation. Hence, the CA can be utilized as a coating agent for the MNPs synthesis [35]. Similarly, Munjal et al. reported CA-coated $CoFe_2O_4$ NPs (CA-$CoFe_2O_4$-NPs) using rapid mechanochemical ligand exchange and oleic acid hydrothermal synthesis (OA-$CoFe_2O_4$). Herein, CA is a suitable stabilizer for OA-$CoFe_2O_4$ NPs. It may because of the presence of plenty of functional groups. CA helps OA-$CoFe_2O_4$ NPs to enhance the solubility/dispersibility in water due to the water-loving nature of CA. In that, the NPs are transformed into hydrophilic NPs by treating with CA via rapid mechanochemical ligand exchange process, which makes $CoFe_2O_4$ NPs stable, colloidal solution in water. Herein, CA is not only offering stability but also is one of the "abundant and easily available" ligands, which can be a good alternative to different types of ligands such as chitosan, PEG, etc. In addition, the short-chain of CA provides a more dense coating on the NPs. Interestingly; these NPs can enhance the super-hydrophilic properties, i.e. water dispersibility of the NPs. Hence, CA-coated NPs offered high dispersibility into the water system, high zeta potential, and moderate saturation magnetization, and high cytocompatibility. Therefore, shortly, large-scale production of biocompatible and water-dispersible magnetic oxide nanoparticles for magnetic hyperthermia applications, variously employed in recent biomedical applications [36] (Fig. 9).

CA-based dendrimers Cefotaxime sodium (CFTX) is a semi-synthetic and broad-spectrum cephalosporin antibiotic, which shows its effect against the treatment of bacterial infections. Investigators theoretically urged to create an optimal formulation, which can mitigate cytotoxicity and boost pharmacokinetic parameters through the successful focusing of bacterial cement. The CFTX pharmacokinetic parameters indicate the half-life (1 h) of the intramuscular (IM) administration of CFTX. Besides, 30% of CFTX shows protein binding and a maximal serum concentration (about 11.7 μg/mL, within 30 min). Therefore, in 2019, Valikala et al. developed pegylated CA-based CFTX-loaded dendrimers (peg-CA-CFTX) using the divergent method. The synthesis emphasizes the development of PEG diacid as the first-generation dendrimer and interacts with different active ingredients, which help generations of dimethyl form amid and thionyl chloride. Particularly, the chlorinated PEG and CA containing hydroxyl group undergoes the esterification and forms the plain form of dendrimers. The developed plan can provide adequate quantities of a product that could be used for investigating the biological activity. Additionally, it shows a better effect against bacteria (Gram-positive, and Gram-negative microorganisms). Herein, the pegylation is helped to the interaction of dendrimers with the surface of bacteria cell which resulted in the lysis if cell. In addition, the dendrimer internalization in bacterial cells has been confirmed by high fluorescence intensity (Fig. 10). It traced the overall hyperbranched structure that may because of the dendrimer's immediate release after cellular component interaction. Therefore, pegylated CA-based CFTX-loaded dendrimers can be a better alternative to the conventionally engaged CFTX-loaded dosages forms [37].

Rao and co-author designed antifungal drug (econazole) loaded CA-based dendrimeric (Econazole-CA) hydrogels. In this study, econazole is involved in the surface or interior of the CA dendrimers completely through either covalent conjugation or electrostatic interaction. In addition, econazole-loaded CA dendrimers demonstrate the anti-fungal activity against (*Candida krusei* and *Candida albicans*). Overall, CA dendrimers are an approach toward refining therapeutic efficiency, solubility, and bioavailability of entrapped drugs. Thus, it may novel carriers for antifungal agent delivery [38]. Namazi and co-authors were developed gold nanoparticles (Au NPs) in the existence of diverse generations of CA-based dendrimers. Interestingly, CA-based dendrimers offer novelty from the monomeric point of interpretation and their simple preparation method. In pain, CA-based dendrimers show the

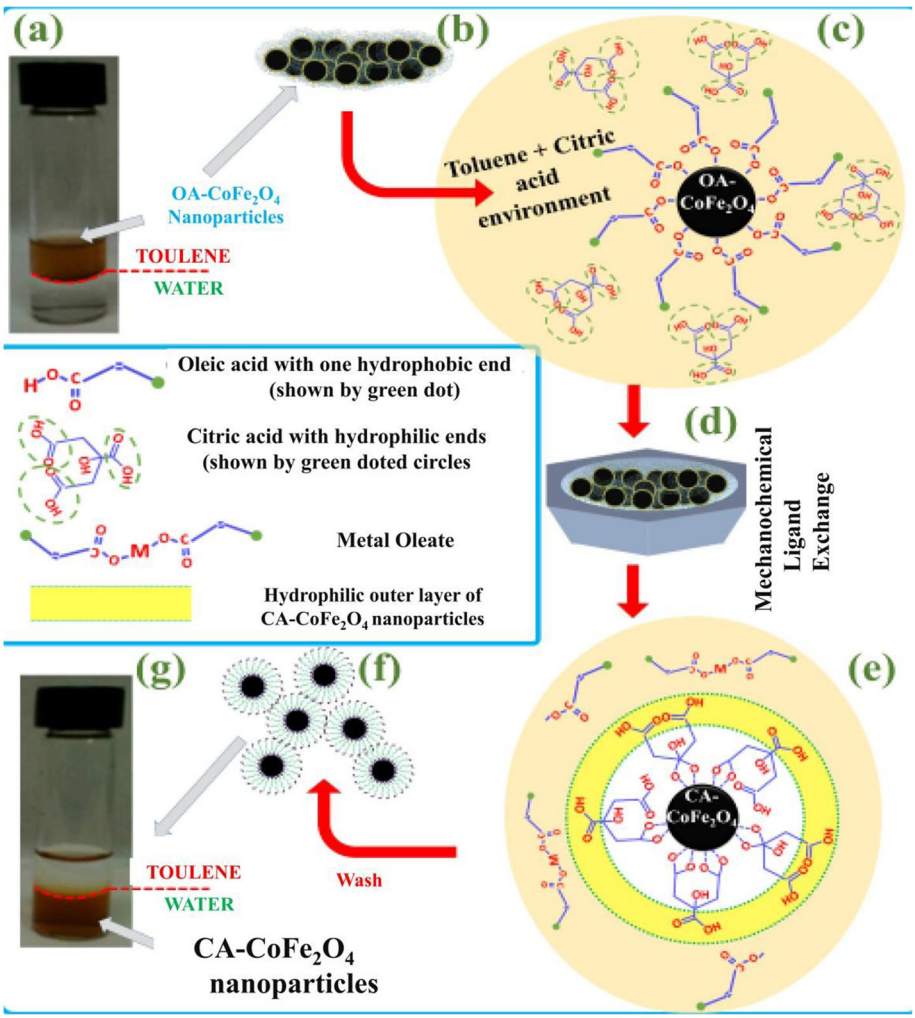

Fig. 9 Schematic presentation of synthesis of CA-coated $CoFe_2O_4$ NPs. The OA-$CoFe_2O_4$-NPs in water and toluene **a**; OA-$CoFe_2O_4$-NPs **b**; OA-$CoFe_2O_4$-NPs in CA and toluene **c**; liquid-based mechanical grinding of OA-$CoFe_2O_4$-NPs **d**; CA coated $CoFe_2O_4$-NPs **e**; CA-Fe_2O_4-NPs **f**; CA-Fe_2O_4-NPs in water and toluene **f**. ["Reprinted (adapted) with permission from [36]. Copyright 2018 Elsevier."]

different surface functionalities at the surface including the hydroxyl group. In the case of the synthesis of AuNPs, the AuNPs interact with the surface containing hydroxyl groups of CA-based dendrimers. It confirmed that the AuNPs complexed with a surface containing periphery hydroxyl groups of CA-based dendrimers. Overall, because of this, CA-based dendrimers gives the stable and uniform AgNPs [39]. Namazi and co-workers have been developed the CA and PEG-based tri-block dendrimers (CA-PEG-CA) that can help as potential delivery systems. In this work, the compound G1 has been synthesized using the chlorinated PEG (ClOC–PEG–COCl) and CA. After that, the Dicyclohexyl carbodiimide has been used for the synthesis of G2, and finally, the G2 along with CA converted the complete G3 dendrimers. Herein, the hydroxyl group of CA and chlorinated PEG resulted in the esterification reaction. Notably, after loading naproxen into dendrimers, the imidazole containing lone pair of electrons gets protonated along with naproxen containing an acidic proton. It may due to the electrostatic interaction among the naproxen acidic group and imidazole nitrogen atom containing lone pair of electrons. It helps to load the drug and regulation of release as compared to the plain dendrimers. Herein, the CA-based dendrimers offer several merits including less toxicity, high water-solubility, and good biocompatibility. Naproxen (lipophilic drug molecule) contains an acidic group that is loaded into functionalized plus non-functionalized dendrimers. Amazingly, the naproxen release rate has been increased in pH 10 in all generations. It may due to an increase in naproxen solubility plus ester bonds hydrolysis in the revealed pH 10. An outcome of this investigation shows that the quantity of the trapped active pharmaceutical

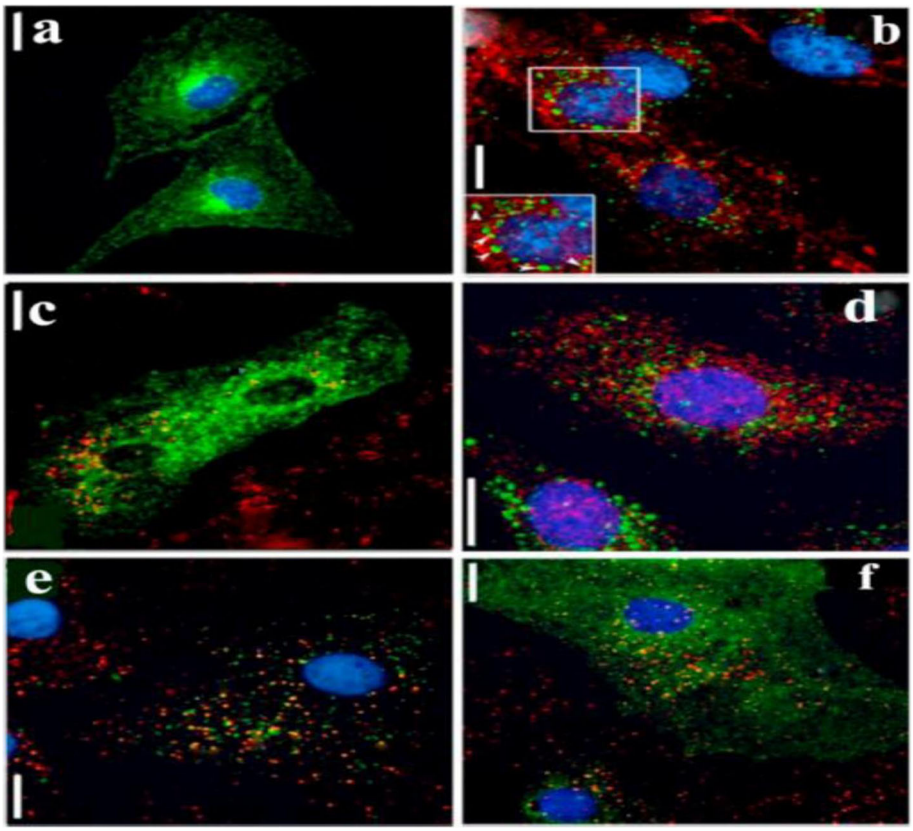

Fig. 10 Confocal microscopic images of pegylated CFTX-dendrimers after 1h incubation in the bacterial perinuclear segment **a**; endosome **b**, localization of fractional CFTX dendrimers in lysosomes **c**, **d**; endosomal localization of dendrimers 10 min of incubation **e**, **f**. ["Reprinted (adapted) with permission from [37]. Copyright 2019 Elsevier."]

ingredient increased along with the increase in the production of the dendrimer plus pH. Hence, CPEGCA triblock dendrimers would be great potential for drug/gene delivery systems [40].

CA-based branched polymer nanoconjugates

In 2018, Mani and co-workers developed the folate-thioglycolate-gold nanoconjugates (FA-TGA-AuNPs) using CA-PEG branched polymer for inhibition of overall MCF-7 cancer cell proliferation. In this study, CA-PEG branched polymer (CPEG) has been used as a reducing agent plus stabilizing agent in AuNPs synthesis. The thiol group (Au-S) of thioglycolic acid (TGA) attached to CPEG-stabilized AuNPs and finally resulted in an association with the free carboxylic acid (COOH) group present on TGA-AuNPs nanoconjugates surface. Overall, CPEG-based TGA-AuNPs nanoconjugate shows good stability as compared to citrate-stabilized AuNPs. Further, the FA attachment onto TGA-AuNPs nanoconjugates (FA-TGA-AuNPs) was accomplished through EDC/NHS coupling reaction-using AuNPs containing the COOH group. Concisely, the FA-TGA AuNPs not only acted as a potential drug targeting agent for 5-fluorouracil (5-FU) but also inhibited the overall growth of MCF-7 cells even in the absence of anticancer drug therapy. This conjugate sustained the release of 5-Fu for up to 5 days. The faster drug release to special targeting of cancer cells is anticipated due to a more acidic tumor microenvironment of cancer cells as compared to the usual cellular setting. Hence, in the future point of view, the biocompatible FA-TGA-AuNPs nanoconjugates could be employed as a substitute active agent delivery system to breast cancer treatment which can help to achieve improved therapeutic efficacy with decreased drug dose [41].

Mahdieh et al. synthesized Fe_3O_4@zinc oxide (ZnO) core @shell NPs retouch on graphene-oxide (GO) grafted with poly-CA (PCA) magnetic quaternary nanocomposites (GO-g-PCA/Fe_3O_4@ZnO). In brief, the biocompatible nanocomposite (GO-g-PCA/Fe_3O_4@ZnO) was completed by Fe_3O_4@ZnO core@shell nanoparticles on GO grafted with PCA using different amounts of GO through the hydrothermal method. The hydroxyl group of PCA and COOH group of GO resulted in the bonding, which gives the grafted compound (GO-g-PCA). The resulting GO-g-PCA/Fe_3O_4@ZnO composite

provides superparamagnetic properties, preserved the crystalline phase of the core and the shell. Moreover, the highest saturation magnetization (~ 54.99 emu/g) was observed by Fe_3O_4@ZnO. Generally, core@shell nanoparticles with GO-g-PCA help to improve the photocatalytic performance. The photocatalytic performance concludes that by increasing the quantity of GO, the overall photocatalytic activity has been improved. In conclusion, the quaternary nanocomposite provides the highest photocatalytic activity. Therefore, it can be used in nanomedical applications because of its biocompatibility [42]. As we know, the CA is a biodegradable, cheap, plus biocompatible compound. It is extensively used in soft drinks, food, cosmetics, detergent, cleaner. It is mainly the primary capping agent for metal nanoparticles. Ample literature survey revealed that the hyperbranched polyglycerol (h-PG) is a promising material for biomedical applications. It may due to its properties including architectures, a large number of functional groups, thermal stability, relatively simple synthesis, and purification, biocompatibility, and low-toxicity. In this content, Bodaghi and co-authors developed PG- based CA-NPs (PG-CA-NPs). In brief, for adenine PG-based PCA copolymer (Ad-PG-PCA) synthesis, PCA, PG, and Ad have been used. It is noticeable that the CA carboxylic groups units developed the bonding with hydroxyl functional groups of adenine-PG (Ad-PG) by a simple esterification method in the occurrence of silica gel and PG-CA-NPs. Because of huge surface functionalities existed into the surface of PG-CA-NPs, it shows the higher cellular uptake and biocompatibility. Owing to the simple and straightforward synthesis approach, it can be used for various future biomedical applications [43]. Yet a similar line of work has been reported by Mani and co-author. The biocompatible CA–PEG hyper-branched polymer (CPHP) using PEG as a heart component and CA is utilized to assemble the backbone of the new CA-PEG linear dendrimer. The hybrid CA–PEG-based macromolecule has been developed wherein CA and PEG go through polymerization. The combination of an original type of aliphatic CPHP along with a π-bond on the polymeric backbone and its water-soluble aggregates induced the green emission behavior. In CPHP, CA is used as a core element to design/build the backbone. In this present work, the PEG (linking agent) helps to connect CA units of two different compounds and result into the CPHP. In addition, the CA containing hydroxyl group condensed with protons of adjacent methylene that further resulted in the π-bond development into the polymer's backbone. In the future prospective, the biocompatible CPHP may find potential applications in various biomedical fields; it can be used as an extracellular matrix for tissue engineering applications [44].

CA as a hyperbranched

Adeli et al. reported the hyperbranched PCA applications in anticancer drug (cisplatin) delivery system. In this study, the prepared hyperbranched polyester has relied on CA and glycerol monomers. In this framework, the condensation of CA (monomer) in the presence of glycerol (monomer) with different CA/glycerol molar feed ratios resulted in the development of hyperbranched poly (CA–glycerol). More specifically, the hyperbranched polyesters with CA and glycerol building blocks could be synthesized through a stepwise melting polycondensation reaction. Due to the huge amount of functional groups on the surface of obtained hyperbranched copolymers, it offers a high drug loading capacity. Therefore, it can be used as a good substitute for the delivery of anticancer drugs with high efficiency. The CA and glycerol-based copolymers containing surface functionalities efficiently interact with the cell membranes and accordingly transfer the active agent across the membrane at a high rate. Particularly, CA shows good water solubility and biocompatibility, and high molecular weights, and therefore, the synthesized hyperbranched polyesters can be used as an auspicious material for biomedical applications [45].

CA as a solvent

Recently, CA has been used as a solvent in polymeric carrier (i.e., pectin obtained from banana peels) for amoxicillin-loaded gastroretentive floating beads. In this pioneered study, the CA has been used as a solvent for degrading the pectin (depolymerizing and de-esterifying). In addition, CA can be utilized to isolate pectin along with better gelling properties. Moreover, the increase in the strength of CA resulted in the rise in pectin yield. The CA provides an admirable degree of esterification containing pectin that resulted in good gel properties to the CA. Moreover, it demonstrates that the pectin can be used as compatible floating drug delivery for amoxicillin. Because of the good gelling properties of pectin, it prolonged the release of amoxicillin in the stomach. Therefore, the CA can be an excellent solvent for the isolation of pectin [46].

CA as a novel disintegrant

Pachuau and co-workers have developed the novel modified taro starch (*Colocasia esculenta*) by treating it with CA. In these attempts, the citrated taro starch has been used as a disintegrant paracetamol tablet. During the synthesis of citrated taro starch, the addition of water into the process material resulted in the swelling. Because of this, the size of modified starch can be increased slightly as compared to the plain taro starch. In addition, the viscosity of taro starch can be modified upon the addition of CA, which may due to the

breakdown of glycosidic linkage of starch. Paracetamol tablet with modified starch shows good mechanical properties such as tensile strength. Furthermore, the citrated taro starch shows a direct relation with disintegration time. It is worthy to mention that the lower hardness and few amounts of moisture content present in modified starch resulted in the higher disintegration efficiency. In addition, this helps for media penetration and finally, it resulted in the disaggregation of the tablet. The overall dissolution efficiency has been increased from 75 to 80% when the citrated taro starch concentration into the tablet increased from 2.5 to 10%. Furthermore, the mean dissolution time (MDT) has been reduced from 7.54 min to about 6 min in citrate taro starch. Hence, a disintegration efficacy study resulted that the taro and citrate-modified taro starch exhibited remarkable tablet disintegrant property as compared to standard cornstarch. In prospects of the starch, CA can be a potential application in pharmaceutical industries [47].

CA as a gas-generating agent and release enhancer

Gastroreductive dosage formulations are more cautious so they can improve the efficiency of controlled floating release systems in drug delivery. A mechanism that persists in the stomach side. It intended to release the drug with an appropriate interval against all physiological barriers. It is ultimately metabolized in the organism may also be described as the best floating controlled release system. Physiological challenges, such as gastric motility and stomach retention. The gas-generating agent helps prolong the residence time of dosage forms, the active is released at the desired rate. Mali and co-authors have reported the quinapril hydrochloride gastroretentive floating tablets using CA as a gas-generating agent. Herein, the contact of dissolution media with CA resulted in the generation of carbon dioxide that helps to float the dosage form on the surface of dissolution media. Interestingly, in vitro quinapril release shown a slower release when the increases the HPMC K4M and CA concentration from 84.659 to 98.234%, respectively. Additionally, optimized formulation (CA concentration 8%) demonstrates satisfactory sustained release (98.234% within 12 h) and it remained buoyant on pH 1.2 for more than 12 h. The lag time range from 94 to 121 s, and percent buoyancy was 98.23%. Gas-generating agents are used for the effervescence in a dosage form, which helps the tablet float in gastric fluid. A high concentration of HPMC K4M and CA sustained the quinapril HCl release from floating tablets. It may due to the tablet floats in gastric fluid the bulk density less than the gastric fluids and so remains buoyant into the stomach without effect in the gastric emptying time for a prolonged period. Overall, the application of CA with proper concentration into a gastro retentive tablet dosage form can provide an acceptable floating profile [48]. In further attempt, Jagdale and co-workers used the design of the experiment for the development of tapentadol hydrochloride tablet floating drug delivery using CA as a gas-producing agent. In brief, formulations tablet containing 15% and 20% of sodium bicarbonate alone failed to float. Whereas, the formulation containing 20% sodium bicarbonate along with 10% CA exhibited good floating with floating lag time 1 min, etc. It may due to the desired amount of carbon dioxide released into tablet formulation upon contact of dissolution medium. Herein, the combination of xanthan gum plus locust bean gum achieved the controlled release of tapentadol hydrochloride. Therefore, in chronic and moderate pain treatment, a floating plus controlled release formulation was used to improve overall bioavailability and therapeutic efficacy [49]. The key to the problem of effervescent products is physical and chemical instability especially during sodium bicarbonate used as a gas-generating agent. It is also reported that oral treatment of gastric disorders with an H_2-receptor antagonist (i.e., ranitidine or famotidine) is used along with a combination with antacids. It promotes the local delivery of these approved drug(s) to the parietal cell wall receptor. Therefore, ranitidine hydrochloride systemic/local delivery can efficiently offer low gastric acid secretion. In 2014, Dave and co-authors have designed sustained-release ranitidine hydrochloride gastroretentive formulation using a low quantity of CA and a high quantity of stearic acid. The generation of gas (carbon dioxide) from sodium bicarbonate upon contact with dissolution media helps to the conversion of dosage form into low-density material as compared to the density of dissolution media. In the fed state stomach (pH ~ 3.5), the CA can be engulfed into providing an acidic medium to sodium bicarbonate. Furthermore, CA offered the stabilizing effect on ranitidine hydrochloride formulations. The hydrophilic nature of CA enhanced the dissolution rate as well as enhanced the release rate of the drug. The addition of stearic acid offers a sustained release of ranitidine hydrochloride that may due to the hydrophobic nature of stearic acid. Overall, the proper balance between a release rate enhancer and retardant can produce a similar drug dissolution profile concerning the theoretical dissolution profile [50].

CA as a stabilizer

Behdadfar et al. developed that the superparamagnetic and monodispersed, aqueous ferrofluids of zinc substituted magnetite NPs ($Zn_xFe_{3-x}O_4$) synthesized using a hydrothermal-reduction route with using CA as a stabilizer and reducing agent. CA was employed as a modulator plus reducing agent into the development of spinel structure as well as controlled nanoparticle size,

crystallinity, etc. In this study, the rising CA amount into the synthesis process resulted in the conversion of hematite to magnetite. Furthermore, the existence of CA helps to the conversation of Fe^{3+} to the Fe^{2+} and finally the formation of magnetite. That entirely depends on the concentration of CA. The efficient binding of CA surface groups to the nanoparticles confirmed the surface potential and accordingly the stability of nanoparticles. It is worthy to mention that the presence of citrate ions on the surface of $Zn_xFe_{3-x}O_4$ assured the long-term stability in water, which was confirmed by the zeta potential of nanoparticles. Zeta potential was measured at diverse pH values between 2 and 12. Whereas, the NPs at medium pH of 7 shows negative surface potentials of around 20 mV for x¼0 and around 25 mV for other NPs. Overall, these citrate stabilized NPs are cheap, simplistic, low energy, and eco-friendly method that leads to the formation of ferrofluids along with high intrinsic loss powers. So, the $Zn_xFe_{3-x}O_4$ NPs preparation can be a great prospective application in magnetic hyperthermia [51].

CA as an absorption enhancer

Gut hormone (Glucagon) is one of the key regulatory elements in glucose homeostasis and is clinically used for the management of hypoglycemia and premedication in peroral endoscopy. The dry powder inhaler (DPI) glucagon form is believed to be an auspicious new dosage form. In 2009, Inoue et al. developed a novel glucagon-DPI by using CA as an absorption enhancer for enhanced pharmacological effects. The dissolution of glucagon is more soluble in acidic media. Therefore, the presence of CA in DPI resulted in excellent dissolution in water. In addition, the water-loving nature of CA might impart the dissolution of DPI. Generally, the CA concentration can degrade the glucagon, which also depends on the temperature conditions. Amazingly, the CA containing DPI shows the good stability of glucagon in terms of an acidic condition associated with degradation. Moreover, the pharmacological activities confirmed the role of CA as an absorption enhancer. Interestingly, the addition of CA resulted in significant progress in glucagon-targeted pharmacological activities in a concentration-dependent order. Concerning the role of CA as an absorption enhancer, various mechanisms have been reported. Principally, CA can lower the intracellular pH, which helps to increase the Ca levels by reducing adenosine triphosphate (ATP) levels in the intracellular acidosis. Then, these biochemical events might lead to increased permeability of the near tight bonding and finally enhanced the transport of the drug by the paracellular route. Another possible mechanism is based on the enzyme's degradation. Notably, the CA helps to reduce the mucosal pH that may be important for the inhibition of glucagon proteolytic degradation by different types of enzymes. In this study, concentration-dependent and time-dependent release of LDH was found in capric acid, but not in CA-treated cells. Newly designed glucagon-DPI shows high dispersion and remarkable deposition into respiratory organs along with an emitted dose and fine particle fraction of 99.5% and 25%, respectively. Moreover, glucose levels in rats have been monitored after inhalation of glucagon-DPIs. In contrary to glucagon-DPI without CA, a momentous improvement for the hyperglycemic effects was found in glucagon-DPI containing CA. According to the AUC values, the addition of CA led to a significant improvement of pharmacological activities of glucagon in a concentration-dependent approach. Moreover, adding CA to glucagon-DPI enhanced dissolution and did not change the stability of the glucagon-DPI in its solid state. Overall, CA is of interest to both pH modifier and absorption enhancer, which has shown that this can increase the dissolution property of glucagon. In the future, there is an urge to initiate clinical trials for the safety and efficacy of CA-based DPI [52]. Taken as a whole, CA is an excellent emerging material in various pharmaceutical and biomedical applications (Table 1).

Conclusion

CA is a pharmaceutical carrier gaining much appeal due to the plenteous merits in comparison with other pharmaceutical excipients. CA is an outstanding material for plentiful pharmaceutical applications because it improves the overall efficacy and safety of the pharmaceuticals. In addition to that, CA-based different modified materials also confirmed the biodegradability and biocompatibility. It is worth mentioning that the CA offers numerous benefits including stable, size-controlled, crystalline nanoparticles synthesis can be possible. Furthermore, CA plays an important role as a reducing agent, coating agent, and modulator in the development of spinel structure and nanocarriers. The literature concludes that CA increased the absorption of the drug and improved the dissolution of active in the targeted side due to the presence of numerous functionalities. An application CA as a gas-generating agent exhibited a good floating profile along with extended drug release. Moreover, it also shows the high drug loading and efficient drug delivery to the targeted side. Due to its hydrophilic nature, CA reduces the disintegration time of tablets. The role of CA as a solvent can offer a low degree of esterification that shows good gelling ability and subsequently retard the release of the drug. Due to several surface functionalities, CA shows a higher cellular uptake and good biocompatibility. In addition, CA in form provides strong fluorescence in several biomedical applications. Furthermore, a review evinced the CA can be

Table 1 Summary of pharmaceutical applications of CA

Sr. No	Application of CA	Active agent	Composite	Result/Inference	Ref.
1.	Green crosslinker	CIFLOX	PVA-CA-AgNPs	1. CA acted as a green crosslinker of PVA by esterification reaction. 2. The number of free carboxyl groups in CA provided pH sensitivity and antimicrobial properties to the PVA.	[14]
2.	Green crosslinker	–	PVA-CA	1. Aqueous stability plus biocompatibility of PVA increased.	[15]
3.	Green crosslinker	Fe_2O_3	Fe_2O_3-CA-MNPs	1. It shows good superparamagnetic behavior. 2. It increased the antibacterial activity of Fe_2O_3.	[16]
4.	Green crosslinker	Ketoconazole	β-CD-HEC-CA	1. It provides the controlled release of ketoconazole.	[17]
5.	Green crosslinker	Diclofenac sodium	CL-MPH	1. It shows the stimuli-responsive zero-order release of the drug.	[18]
6.	Green crosslinker	–	CA-WPI	1. It confirmed that the CA is a novel gelling agent.	[19]
7.	Green crosslinker	–	WPI-CA	1. The crosslinking of WPI-CA increased the overall gel water holding capacity 2. CA interlinking resulted in a much more digestibility decrease than swelling.	[21]
8.	Green crosslinker	–	WPI-CA	1. It resulted in higher water-holding capacity plus denser microstructure.	[22]
9.	Green crosslinker	MB, Tetracycline	NaCMC-HPMC	1. It provides the sustained release of the drug (up to 72 h). 2. It demonstrated significant antibacterial activity.	[11]
10.	Green crosslinker	–	Xanthan-CA hydrogel	1. The cross-linking decreases the swelling of the hydrogels whereas improving their structure to stabilize the products when submerged in water.	[23]
11.	Green crosslinker	Tramadol	PEGD: CA/GEL	1. It provides the low-cost 3D hydrogel with an anomalous drug transport mechanism for the release of tramadol.	[23]
12.	Green crosslinker	MB	CMC-PEO	1. It provides the pH-dependent release of MB.	[24]
13.	Green crosslinker	Ketoconazole	CMC-PEG hydrogel	1. It provides the controlled release of ketoconazole.	[17]
14.	Green crosslinker	Moxifloxacin hydrochloride	CMTG-CA	1. Prepared biocompatible hydrogel provides the controlled release of drugs along with a non-Fickian drug transport mechanism.	[25]
15.	Green crosslinker	Levofloxacin	Starch-CA	1. The reduced level of matrix hydration resulted in a long-time release of levofloxacin.	[26]
16.	Green crosslinker	Cefixime	CA-Ag-Gelatine	1. It demonstrates the pH-dependent swelling of hydrogel and release of the drug.	[27]
17.	Co-crystal	Effervescent product	CA-NIC	1. CA improves eeffervescent product stability.	[28]
18.	pH modifier	SBA-1	SBA-15CTA	1. It confirmed that the CA containing two carboxyl groups is more effective for the development of high-quality SBA.	[29]
19.	pH modifier	AZA	AZA@CA	1. The inclusion of CA increased the basic amino group's ionization in AZA. 2. It increased its release in dissolution colonic pH.	[30]
20.	Fluorescent material	–	TPCA	1. It provides strong fluorescence. 2. It shows the selectivity toward interest analyte.	[30]
21.	Fluorescent material	siRNA	PPFR	1. PPFR exhibited stable photoluminescent aptitude. 2. It successfully binds and guards the siRNA against RNase.	[31]
22.	Fluorescent material	–	CA-GQDs	1. It synthesized stable blue luminescent GQDs.	[32]
23.	Fluorescent material	DOX	CA-CDs-DOX	1. It shows the higher cellular uptake plus good anti-tumor potential	[33]
24.	Capping agent	Quercetin	CA/α-CD-IONPs	1. It provides the stable IONPs 2. It shows the pH-dependent release of quercetin.	[34]
25.	Capping agent	$Zn_{0.3}Fe_{2.7}O_4$ NPs	PEG-$Zn_{0.3}Fe_{2.7}O_4$ NPs	1. It gives non-toxic and stable nanoparticles.	[35]
26.	Coating agent	CA-$CoFe_2O_4$-NPs	CA-$CoFe_2O_4$-NPs	1. It gives the stable and biocompatible NPs.	[36]
27.	Dendrimers	CFTX	Peg-CA-CFTX	1. It provides sustained release (48 h) and good antibacterial activity against Gram-negative and Gram-positive bacteria.	[37]
28.	Dendrimers	Econazole	Econazole-CA-	1. It shows excellent antifungal activity.	[38]

Table 1 Summary of pharmaceutical applications of CA *(Continued)*

Sr. No	Application of CA	Active agent	Composite	Result/Inference	Ref.
			dendrimers		
29.	Dendrimers	AuNPs	CA-dendrimers	1. It synthesized the CA-based dendrimers gives stable and uniform AgNPs.	[40]
30.	Dendrimers	Naproxen	CA-PEG-CA	1. It provides the high loading of naproxen followed by increasing the release of naproxen.	[40]
31.	Polymeric conjugates	5-FU	FA-TGA AuNPs	1. It provides the targeted and sustained drug release of 5-FU. 2. FA-TGA AuNPs nanoconjugates increased the cell proliferation of normal cells without toxicity.	[41]
32.	Polymeric conjugates	–	GO-g-PCA/Fe_3O_4@ZnO	1. It improved the overall photocatalytic activity of nanocomposite.	[42]
33.	Polymeric conjugates	Cy5-dye (as a model)	PG-CA-NPs	1. It shows a higher cellular uptake and good biocompatibility.	[43]
34.	Polymeric conjugates	–	CPHP	1. It develops the water-soluble polymeric conjugates. 2. It gives more than 80% cell viability.	[44]
35.	Hyperbranched polymer	Cisplatin	CA–glycerol	1. It shows the high loading of cisplatin and efficient delivery of the same. 2. It shows a lower IC50 value than the pure anticancer drug, which confirmed the biocompatibility of copolymers.	[45]
36.	Solvent	Amoxicillin	Pectin	1. It helps to synthesize the pectin with low degree esterification. 2. Owing to low degree esterification, it shows good gelling ability and subsequently retard the release of the drug.	[46]
37.	Superdisnitegrant	Paracetamol	Citrated taro starch	1. It reduces the disintegration time of tablets. 2. It enhances the dissolution efficiency of the tablet.	[47]
38.	Gas generating agent	Quinapril hydrochloride	–	1. It shows a good floating profile. 2. It gives the sustained release of the drug for 12 h.	[48]
39.	Gas generating agent	Tapentadol hydrochloride	–	1. It exhibited the good floating profile 2. It provides the controlled release of tapentadol hydrochloride.	[49]
40.	Release enhancer	Ranitidine hydrochloride	–	1. The less concentration of CA and high concentration of stearic acid resulted in sustained release of ranitidine. 2. The addition of CA into dosage form enhanced the release rate of the drug.	[50]
41.	Stabilizer	–	$Zn_xFe_{3-x}O_4$ NPs	1. CA offers stable, size-controlled, crystalline nanoparticles. 2. CA plays an important role as a reducing agent and modulation in the development of spinel structure.	[51]
42.	Absorption enhancer	Glucagon	Glucagon-DPI	1. It increased the absorption of glucagon in DPI. 2. It improved the dissolution of glucagon in the targeted side.	[52]

used in biomedical applications as a fluorescent material (CDs/GQDs), that can be used for the in vitro diagnosis of several life-threatening agents. Overall, CA-based composites are appealing pharmaceutical carriers, which modify the drug release with the desired rate and can be employed for targeted drug delivery. This perspective of CA in pharmaceuticals relies on noticeable features such as biophysical, biochemical, etc. As the CA novel applications are up till now at a laboratory scale only, therefore additional research is essential to employ them as large commercial applications. Further research is also needed to enhance the benefits of interesting and flexible characteristics such as the potential to create a pioneering material in applications of CA in drug delivery systems. Finally, it could be concluded that CA is an extremely promising material for pharmaceutical applications.

Abbreviations

CA: Citric acid; GRAS: Generally recognized as a safe; FDA: Food and Drug Administration; PVA: Polyvinyl alcohol; AgNPs: Silver nanoparticles; PVA-CA-AgNPs: PVA/CA/AgNPs based conjugates; CIFLOX: Ciprofloxacin; *S. aureus*: *Staphylococcus aureus*; Fe_2O_3: Iron oxide; MNPs: Magnetic nanoparticles; NPs: Nanoparticles; β-CD: β-cyclodextrin; β-CD-HPMC: β-CD grafted hydroxypropyl methylcellulose; MP: *Mimosa pudica*; CL-MPH: Cross-linked MP hydrogel; WPI: Whey protein isolate; CA-WPI: CA-mediated food protein; Na-CMC: Sodium carboxymethylcellulose; HPMC: Hydroxypropylmethylcellulose; NLC: Nanostructured lipid carriers; WHC: Water holding capacity; MB: Methylene blue; PBS: Phosphate buffer saline; PEG-D: Poly (ethylene glycol) diamine; GTA: Glutaraldehyde; GEL: Gelatine; PEO: Polyethylene oxide; PMC: CMC/PEO composite; CMTG: Carboxymethyl tamarind gum; ATR-FTIR: Attenuated total reflection-Fourier transform infrared spectroscopy; CA-Ag-Gelatine: Gelatine/CA/Ag nanocomposite; SBC: Sodium bicarbonate; NIC: Nicotinamide; SBA-15: Mesoporous silica; COOH: Carboxy groups; AZA: Azathioprine; QY: Quantum yield; TPCA: 5-Oxo-3,5-dihydro-2H-thiazolo[3,2-a]pyridine-7-carboxylic; CDs: Carbon dots; SMFs: Small molecular fluorophores; CA-CDs: CA-based CDs; PPFR: Poly (CA)-polyamine conjugated with FA and rhodamine B; CA-GQDs: CA-graphene quantum dots; DOX: Doxorubicin; CDs-DOX: DOX with CDs; α-CD: α-Cyclodextrin; IONPs: Fe_2O_3 NPs; CA/α-CD-

IONPs: CA/α-CD functionalized IONPs; DFT: Dry film thickness; PEG-$Zn_{0-3}Fe_{2-7}O_4$ NPs: PEG-coated $Zn_{0-3}Fe_{2-7}O_4$ NPs; CA-$CoFe_2O_4$-NPs: CA coated $CoFe_2O_4$ NPs; OA-$CoFe_2O_4$: Oleic acid-$CoFe_2O_4$; CFTX: Cefotaxime sodium; CA-PEG-CA: CA and PEG-based tri-block dendrimers; ClOC–PEG–COCl: Chlorinated PEG; Au NPs: Gold nanoparticles; CPEG: CA-PEG branched polymer; FA-TGA-AuNPs: Folate-thioglycolate-gold nanoconjugates; TGA: Thioglycolic acid; FA-TGA-AuNPs: FA attachment onto TGA AuNPs; 5-FU: 5-fluorouracil; ZnO: Zinc oxide; GO: Graphene-oxide; PCA: Poly-CA; h-PG: Hyper-branched polyglycerol; Ad-PG-PCA: PG-based PCA copolymer; Ad-PG: Adenine-PG; CPHP: CA–PEG hyper-branched polymer; MDT: Mean dissolution time; $Zn_xFe_{3-x}O_4$: Ferrofluids of zinc substituted magnetite NPs; DPI: Dry powder inhaler; ATP: Adenosine triphosphate

Acknowledgements
The authors are thankful to the institute for providing facilities such as library, journals, and internet sources for writing the article.

Authors' contributions
SN: conceptualization, methodology, writing—original draft, writing—review and editing, visualization; YV: writing—original draft, writing—review and editing; RS: writing—original draft, writing—review and editing; SD: writing–original draft, writing—review and editing; PP: writing—original draft, writing—review and editing. All authors gave their individual critical revision and final approval of the version to be submitted. The authors read and approved the final manuscript.

Funding
Not applicable.

Availability of data and materials
All data and materials are available on request.

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no conflict of interest.

Author details
[1]Department of Pharmaceutical Chemistry, H. R. Patel Institute of Pharmaceutical Education and Research, Shirpur, Dist-Dhule 425405, India. [2]Department of Pharmaceutics, H. R. Patel Institute of Pharmaceutical Education and Research, Shirpur, Dist-Dhule 425405, India. [3]Department of Pharmaceutical Science and Technology, Institute of Chemical Technology, Matunga, Mumbai, MS 400019, India.

Received: 2 December 2020 Accepted: 8 February 2021
Published online: 25 February 2021

References

1. Soccol CR, Vandenberghe LP, Rodrigues C, Pandey A (2006) New perspectives for citric acid production and application. Food Technol Biotechnol 44(2):141–149
2. Swain MR, Ray RC, Patra J (2011) Citric acid: microbial production and applications in food and pharmaceutical industries. Citric Acid:97–118
3. Thauer RK (1988) Citric-acid cycle, 50 years on: modifications and an alternative pathway in anaerobic bacteria. Eur J Biochem1 76:497–508
4. Salihu R, Abd Razak SI, Zawawi NA, Kadir MRA, Ismail NI, Jusoh N, Mohamad MR, Nayan NHM (2021) Citric Acid: A Green Cross-linker of Biomaterials for Biomedical Applications. Eur Polym J 146(5):110271
5. Lazar Z, Walczak E, Robak M (2011) Simultaneous production of citric acid and invertase by Yarrowia lipolytica SUC+ transformants. Bioresour Technol 102:6982–6989
6. Verhoff FH, Bauweleers H (2000) Citric acid. Ullmann's Encyclop Indust Chem:1–11
7. Ciriminna R, Meneguzzo F, Delisi R, Pagliaro M (2017) Citric acid: emerging applications of key biotechnology industrial product. Chem Cent J 11:22
8. Ma C, Gerhard E, Lu D, Yang J (2018) Citrate chemistry and biology for biomaterials design. Biomaterials 178:383–400
9. Tran RT, Yang J, Ameer GA (2015) Citrate-based biomaterials and their applications in regenerative engineering. Annu Rev Mater Res 45:277–310
10. Vranova V, Rejsek K, Formanek P (2013)Aliphatic, cyclic, and aromatic organic acids, vitamins, and carbohydrates in soil: a review. Sci World J 2013. https://doi.org/10.1155/2013/524239.
11. Dharmalingam K, Anandalakshmi R (2019) Fabrication, characterization and drug loading efficiency of citric acid crosslinked NaCMC-HPMC hydrogel films for wound healing drug delivery applications. Int J Biol Macromol 134:815–829
12. Vacanti JP, Langer R (1999) Tissue engineering: the design and fabrication of living replacement devices for surgical reconstruction and transplantation. The lancet 354:S32–S34
13. Nangare S, Dugam S, Patil P, Tade R, Jadhav N (2020) Silk industry waste protein: isolation, purification and fabrication of electrospun silk protein nanofibers as a possible nanocarrier for floating drug delivery. Nanotechnology 32(3):035101
14. Sabzi M, Afshari MJ, Babaahmadi M, Shafagh N (2020) pH-dependent swelling and antibiotic release from citric acid crosslinked poly (vinyl alcohol)(PVA)/nano silver hydrogels. Colloids Surf B Biointerfaces 188:110757
15. Nataraj D, Reddy R, Reddy N (2020) Crosslinking electrospun poly (vinyl) alcohol fibers with citric acid to impart aqueous stability for medical applications. Eur Polym J 124:109484
16. Khan S, Shah ZH, Riaz S, Ahmad N, Islam S, Raza MA, Naseem S (2020) Antimicrobial activity of citric acid functionalized iron oxide nanoparticles–Superparamagnetic effect. Ceramics International 46(8):10942–10951
17. Ghorpade VS, Yadav AV, Dias RJ, Mali KK, Pargaonkar SS, Shinde PV, Dhane NS (2018) Citric acid crosslinked carboxymethylcellulose-poly (ethylene glycol) hydrogel films for delivery of poorly soluble drugs. Int J Biol Macromol 118:783–791
18. Hussain MA, Rana AI, Haseeb MT, Muhammad G, Kiran L (2020) Citric acid cross-linked glucuronoxylans: A pH-sensitive polysaccharide material for responsive swelling-deswelling vs various biomimetic stimuli and zero-order drug release. J Drug Deliv Sci Tec 55:101470
19. Mohammadian M, Salami M, Emam-Djomeh Z, Momen S, Moosavi-Movahedi AA (2018) Gelation of oil-in-water emulsions stabilized by heat-denatured and nanofibrillated whey proteins through ion bridging or citric acid-mediated cross-linking. Int J Bol Macromol 120:2247–2258
20. Demitri C, Del Sole R, Scalera F, Sannino A, Vasapollo G, Maffezzoli A, Ambrosio L, Nicolais L (2008) Novel superabsorbent cellulose-based hydrogels crosslinked with citric acid. J Appl Polym Sci 110:2453–2460
21. Hashemi B, Madadlou A, Salami M (2017) Functional and in vitro gastric digestibility of the whey protein hydrogel loaded with nanostructured lipid carriers and gelled via citric acid-mediated crosslinking. Food Chem 237:23–29
22. Farjami T, Madadlou A, Labbafi M (2015) Characteristics of the bulk hydrogels made of the citric acid cross-linked whey protein microgels. Food Hydrocoll 50:159–165
23. Simões BM, Cagnin C, Yamashita F, Olivato JB, Garcia PS, de Oliveira SM, Grossmann MVE (2020) Citric acid as crosslinking agent in starch/xanthan gum hydrogels produced by extrusion and thermopressing. LWT 125:108950
24. Kanafi NM, Rahman NA, Rosdi NH (2019) Citric acid cross-linking of highly porous carboxymethyl cellulose/poly (ethylene oxide) composite hydrogel films for controlled release applications. 7: 721-731.
25. Mali KK, Dhawale SC, Dias RJ (2017) Synthesis and characterization of hydrogel films of carboxymethyl tamarind gum using citric acid. Int J Biol Macromol 105:463–470
26. Uliniuc A, Hamaide T, Popa M, Băcăiță S (2013) Modified starch-based hydrogels cross-linked with citric acid and their use as drug delivery systems for levofloxacin. Soft Matter 11:483–493
27. Shafagh N, Sabzi M, Afshari MJ (2018) Development of pH-sensitive and antibacterial gelatin/citric acid/Ag nanocomposite hydrogels with potential for biomedical applications. J Poly Res 25:259
28. Pagire AK, Seaton CC, Paradkar A (2020) Improving stability of effervescent products by co-crystal formation: A novel application of crystal engineered citric acid. Cryst Growth Des 20(7):4839–4844
29. Li L, Liu D, Guo Z, Liu Y, Chu W (2020) Improved facile synthesis of mesoporous SBA-15-CTA using citric acid under mild conditions. J Solid State Chem 282:121079

30. Nath B, Nath LK (2013) Studies on stercuia gum formulations in the form of osmotic core tablet for colon-specific drug delivery of azathioprine. PDA J Pharm Sci Technol 67:172–184
31. Wang M, Guo Y, Xue Y, Niu W, Chen M, Ma PX, Lei B (2019) Engineering multifunctional bioactive citric acid-based nanovectors for intrinsical targeted tumor imaging and specific siRNA gene delivery in vitro/in vivo. Biomater 199:10–21
32. More MP, Lohar PH, Patil AG, Patil PO, Deshmukh PK (2018) Controlled synthesis of blue luminescent graphene quantum dots from carbonized citric acid: Assessment of methodology, stability, and fluorescence in an aqueous environment. Mater Chem Phys 220:11–22
33. Kong T, Hao L, Wei Y, Cai X, Zhu B (2018) Doxorubicin conjugated carbon dots as a drug delivery system for human breast cancer therapy. Cell proliferation 51:e12488
34. Ghafelehbashi R, Yaraki MT, Saremi LH, Lajevardi A, Haratian M, Astinchap B, Rashidi AM, Moradian R (2020) A pH-responsive citric-acid/α-cyclodextrin-functionalized Fe3O4 nanoparticles as a nanocarrier for quercetin: An experimental and DFT study. Mater Sci Eng C109:110597
35. Zargar T, Kermanpur A, Labbaf A, Houreh AB, Esfahani MN (2018) PEG coated Zn0. 3Fe2. 7O4 nanoparticles in the presence of< alpha> Fe2O3 phase synthesized by citric acid assisted hydrothermal reduction process for magnetic hyperthermia applications. Mater Chem Phy 212:432–439
36. Munjal S, Khare N, Sivakumar B, Sakthikumar DN (2019) Citric acid coated $CoFe_2O_4$ nanoparticles transformed through rapid mechanochemical ligand exchange for efficient magnetic hyperthermia applications. J. Magn Magn Mater 477:388–395
37. Valikala V, Santhakumar I, Kannappan K (2019) Synthesis and effect of pegylation on citric acid dendritic nano architectures anchored with cefotaxime sodium. J Photochem Photobiol. B 201:111683
38. Rao BN, Fathima SR, Viswanath V, Prakash KG, Padmini DS, Reddy PS (2016) Novel citric acid dendritic hydrogels for the delivery of econazole nitrate and its antifungal activity. J Appl Pharm Sci 6:094–102
39. Reddy N, Jiang Q, Yang Y (2012) Preparation and properties of peanut protein films crosslinked with citric acid. Ind Crops Prod 39:26–30
40. Namazi H, Fard AMP (2011) Preparation of gold nanoparticles in the presence of citric acid-based dendrimers containing periphery hydroxyl groups. Mater Chem Phys 129:189–194
41. Mani G, Kim S, Kim K (2018) Development of folate-thioglycolate-gold nanoconjugates by using citric acid-peg branched polymer for inhibition of MCF-7 cancer cell proliferation. Biomacromolecules 19:3257–3267
42. Dehghani-Dashtabi M, Hekmatara H, Seyed-Yazdi J (2019) Synthesis and improved photoactivity of magnetic quaternary nanocomposites consisting of Fe3O4@ ZnO core@ shell nanoparticles decorated on graphene-oxide grafted poly-citric acid. Physica B Condens Matter 553:11–17
43. Bodaghi A, Adeli M, Dadkhahtehrani A, Tu Z (2017) Synthesis of polyglycerol-citric acid nanoparticles as biocompatible vectors for biomedical applications. J Mol Liq 242:53–58
44. Mani G, Kyobum K, Sengottuvelan B (2017) Water Soluble self-aggregates induced green emission of biocompatible citric acid-PEG hyper branched polymer. Sci Rep 7:1–7
45. Adeli M, Rasoulian B, Saadatmehr F, Zabihi F (2013) Hyperbranched poly (citric acid) and its application as anticancer drug delivery system. J Appl Polym Sci 129:3665–3671
46. Hendrika Y, Reveny J (2018) Formulation and *in vitro* evaluation of gastroretentive floating beads of amoxicillin using pectin from banana peel (Musa Balbisiana ABB). Asian J Pharm Clin Res 11(3):72–77
47. Pachuau L, Dutta RS, Devi TB, Deka D, Hauzel L (2018) Taro starch (Colocasia esculenta) and citric acid modified taro starch as tablet disintegrating agents. Int J Biol Macromol 118:397–405
48. Mali AD, Bathe RS (2017) Development and evaluation of gastroretentive floating tablets of a quinapril HCl by direct compression technique. Int J Pharm Pharm Sci:935–946
49. Jagdale SC, Patil S, Kuchekar BS (2013) Application of design of experiment for floating drug delivery of tapentadol hydrochloride. 2013 (2013).
50. Dave BS, Amin AF, Patel MM (2004) Gastroretentive drug delivery system of ranitidine hydrochloride: formulation and in vitro evaluation. Aaps PharmSciTech 5:77–82
51. Behdadfar B, Kermanpur A, Sadeghi-Aliabadi H, del Puerto MM, Mozaffari M (2012) Synthesis of aqueous ferrofluids of ZnxFe3− xO4 nanoparticles by citric acid assisted hydrothermal-reduction route for magnetic hyperthermia applications. J Magn Magn Mater 324:2211–2217
52. Onoue S, Yamamoto K, Kawabata Y, Hirose M, Mizumoto T, Yamada S (2009) Novel dry powder inhaler formulation of glucagon with addition of citric acid for enhanced pulmonary delivery. Int J Pharm 382:144–150

Publisher's Note