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Review article

# In situ gelling drug delivery systems for topical drug delivery

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ABSTRACT

In situ gelling formulations are drug delivery systems which typically exist in a liquid form at room temperature and change into gel state after application to the body in response to various stimuli such as changes in temperature, pH and ionic composition. Their biomedical application can further be improved by incorporating drug nanoparticles into in situ gelling systems in order to prolong drug release, reduce dosing frequency and improve therapeutic outcomes of patients, developing highly functional but challenging dosage forms. The composition of in situ gelling formulations influence factors relating to performance such as their syringeability, rheology, drug release profile and drug bioavailability at target sites, amongst other factors. The inclusion of mucoadhesive polymeric constituents into in situ gelling formulations has also been explored to ensure that the therapeutic agents are retained at target site for extended period of time. This review article will discuss traditional techniques (water bath-based vial inversion and viscometry) as well as advanced methodology (rheometry, differential scanning calorimetry, Small Angle Neutron Scattering, Small Angle X-ray Scattering, etc.) for evaluating in situ gel forming systems for topical drug delivery. The clinical properties of in situ gelling systems that have been studied for potential biomedical applications over the last ten years will be reviewed to highlight current knowledge in the performance of these systems. Formulation issues that have slowed the translation of some promising drug formulations from the research laboratory to the clinic will also be detailed.

## 1. Introduction

In situ gelling systems are liquid formulations which form a solid-like depot after injection into the body or application to a topical site [1]. They have increasingly gained attention over the last two decades as an attractive class of responsive drug delivery systems for various pharmaceutical and biomedical applications. They are preferred to conventional injectable depot systems such as wafers or implants because they can be easily prepared with lower production costs, as they: are injectable using smaller gauge needles; could be self-administered using autoregulators; facilitate controlled release of incorporated therapeutic agents, thereby reducing dosing frequency, preventing therapeutic failure or undesirable side effects [2]. Topical administration using these systems is also attractive where the low viscosity state at ambient conditions aids administration and allows processes such as spraying, but the gel state is retentive and controls delivery.

The sol–gel phase transition behavior exhibited by in situ gelling formulations depends on one or a combination of different stimuli, such as pH change, temperature modulation, solvent exchange, ultra violet irradiation and the presence of specific ions or molecules [3]. Typically, this gelation is achieved with polymer solutions, though some inorganic [4] and small organic [5] systems exist, and indeed are gaining interest. In polymer systems, molecular weight (MW), architecture, as well as the temperature and composition of the physiological medium influence the gelation of in situ gelling formulations [6]. This article will concentrate on temperature, ion and pH-sensitive formulations of polymer systems which are attractive for delivery to the body.

Over the last decade, there has been progresses made in the development of techniques that can efficiently define the rheological properties of in situ gelling systems. Traditional techniques include vial/tube inversion techniques and viscometry at physiological temperatures (35–37 °C) and conditions (physiological ionic strength and pH) while advanced methods of evaluating the rheological behavior of in situ gelling systems include oscillatory rheometry [1], which can be paired with characterisational techniques such as differential scanning calorimetry [7], small angle neutron scattering [8], and small angle x-ray scattering techniques to unpick complex phenomena [9] (Fig. 1). The stimulus and concentration-dependent aggregation behaviour of in situ

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gelling formulations have been used as an additional method of studying their rheological properties [10]. Shear rheology can probe changes in viscosity with temperature (Fig. 1a), whilst oscillatory rheology can further detail the viscoelastic nature of the materials (Fig. 1b). SAXS/SANS allows study of nanoscale changes in structure where these gel states are often imparted by supracolloidal assemblies within the solutions (Fig. 1c). Rheo-SANS can also further probe how these nanostructures are affected by shear (Fig. 1d).

In situ gelation is typically imparted by a single polymer, however formulation with additional excipients allows precision manipulation of gel properties and secondary effects which may be beneficial to performance, such as incorporation of biocompatible mucoadhesive polymers [6]. Mucoadhesive polymers facilitate retention of the dosage form on mucosal surfaces, prolong drug residence time and thus improve the therapeutic outcomes of patients [12]. Examples of such mucoadhesive polymers include carbopol 934P, chitosan, sodium carboxymethyl cellulose, hydroxypropyl methylcellulose (HPMC), hydroxypropyl cellulose, and methyl cellulose [13]. Furthermore, additives may impart the required rheological behaviour in either sol or gel state. For example, in situ gelling formulation can be designed such that it exhibits a pseudoplastic behavior which may facilitate stabilisation of dispersed phases, enabling easy removal from a container with shear. Additional examples of additives include co-solvents, dispersed phases and salts which can allow further tailoring of formulation properties [13–14].

Recent reviews are available pertaining to in situ gelling or nanoparticulate gel formulations intended for buccal [16], ocular [17], nasal [18], and vaginal routes of administration [18–22]. Vigani et al reviewed in situ gelling systems for varieties of non-parenteral in situ gelling drug carriers [6]. However, in situ gelling formulations delivered to the cervical cavity as well as the rheological properties of the non-parenteral in situ gelling systems was not reviewed. This current review updates to the current state-of-the-art and expands into fundamental rheological considerations for the gelators. Solid in situ gelling polymeric films [71], gastrointestinal in situ gelling formulations [72,73], and thermoresponsive emulsions [15] were not discussed in this article due to the fundamental difference in the phase change.

This article aims to provide an update on in situ gelators, critically discussing advances in in situ gelling delivery systems that have been investigated over the last decade. The various types of in situ gelling systems will be detailed as well as the traditional and advanced techniques for investigating the rheological and nanoscale behavior of in situ gelling systems.

## 2. Classes of in situ gelling systems

In situ gelling formulations are most commonly reported based on three main types of stimuli; temperature, ion and pH sensitivity. Other possible stimuli include light and redox potential, but these are less commonly utilized for pharmaceutical applications and are not contained within this review. Furthermore, in situ gel formation may be achieved by mixing of reagents for covalent gel formation upon application [24], which is outside of the scope of this review.

### 2.1. Temperature sensitive systems

Thermosensitive in situ gelling systems are typically single phase, sol-like systems in aqueous medium below the lower critical solution temperature (LCST). Above LCST, the entropy of mixing becomes unfavourable causing increased hydrophobic interactions, fast dehydration of the solvated polymer chains, resulting in polymer–polymer interactions which can lead to sol–gel transition [1]. Such systems also display thermoreversible behavior as they could transform into the sol state at temperatures less than the LCST, but become solid-like above LCST. Not all polymers which exhibit LCSTs allow gel formation, and

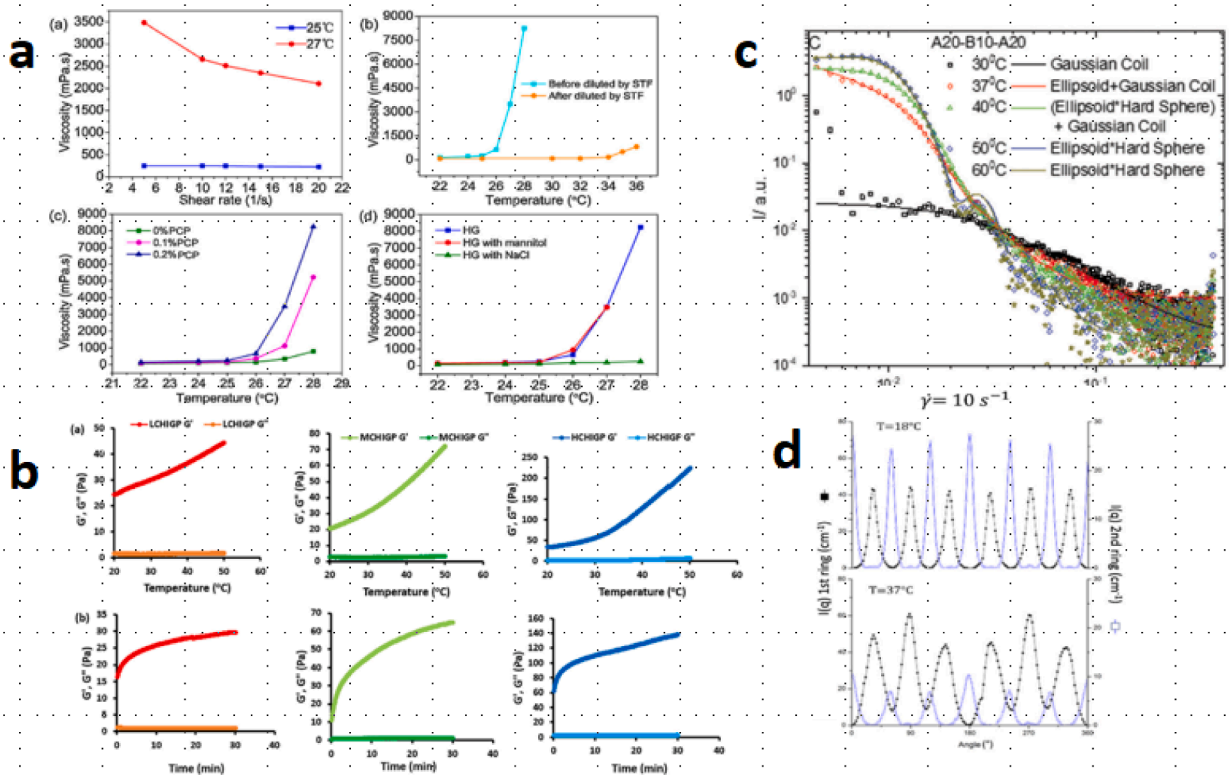


**Fig. 1.** Advances in Rheological techniques (a) Viscosity profile of betaxolol hydrochloride ophthalmic thermosensitive gels studied at various shear rates, temperature ranges and influence of additives [11]; (b) Temperature and time dependent Rheological profile of chitosan-beta-glycerophosphate gels prepared using three chitosan grades [1]; (c) SAXS profile of 5 % w/v PDEA (20 kDA)-PEG (10 kDa)-PDEA (20 kDa) studied from 30 °C to 60 °C [9], and (d) rheo-SANS azimuthal scattering profiles for 25 % w/v F127 at 18 °C and 37 °C under 10 $s^{-1}$ [8]; all images reproduced with permission.

typically homopolymers do not exhibit this phenomenon, for example poly (N-isopropyl acrylamide) polymers typically transition to a cloudy globular state in water when heated [25]. A reliable method to produce thermoreversible gelators is to synthesise copolymers with a hydrophilic co-monomer in addition to the LCST species such that heating results in an amphiphilic state, exemplified by the poly (ethylene oxide)-b-poly (propylene oxide)-b-poly (ethylene oxide) class ("poloxamers") of co-polymers. The amphiphilic polymers may form self-assembled micelles in water at concentrations higher than its critical micellar concentration. For some poloxamers, as the temperature of the amphiphile rises above its critical micellar temperature, the micelles assume an ordered packaging, resulting in gelation [6]. The temperature that gelation occurs at may deviate from precise LCST dependent on the mechanism of gel formation, and this transition temperature is designated as *Tgel*. Other delivery systems have one of the components acting as the cross-linking agent that facilitate sol–gel transition as the temperature of the drug carrier is increased until their gelation temperatures are attained [2].

Rheological evaluation of thermoreversible gels is challenging. The storage modulus (G′) and loss modulus (G″) are rheological terms that define the elastic and viscous behavior of materials, respectively. Elastic materials recover their structure from applied deformation whilst viscous materials resist the flow without recovery [26]. Temperature ramp test and time sweep test are respectively carried out to evaluate the gelation temperature and time of the in-situ gelling formulations. It is expected that samples at $\leq$ 25 °C remain in their low viscosity state during storage and prior to administration in order to facilitate its syringeability. Afterwards, the drug formulation gradually transforms to its gel state in situ at the site temperature to ensure that it is retained on sites for extended period of time; facilitating cellular drug uptake into diseased tissues, and reducing dosing frequency [1].

Temperature ramp analysis is typically carried out by placing a few milliliters of samples directly on a rheometer plate and the plate is heated to the temperature of interest, which simulates the physiological conditions of the target organ [1]. For example, the temperature of the rheometer plate used to study drug formulations intended for ocular and intravesical delivery should be set at 35 °C and 37 °C, respectively [1,7]. During a time sweep test, the time-dependent gelation of drug formulations is typically evaluated at physiological temperature. The magnitude of frequency and strain used for rheological studies should remain within the linear viscoelastic region of the in situ gelling materials so that the micro-structural properties of the sample is not destroyed [27].

Temperature ramps typically identify gelation temperature as point where a sample transitions from predominantly "liquid-like" (G″ > G′) to predominantly "solid-like" (G′ > G″) [26–29]. Alternatively, the gelation temperature may be identified as the temperature where there is an increased growth rate of G′ relative to the G″ without samples necessarily displaying a cross-over of G′ and G″ [2,5]. Nevertheless, some researchers have acknowledged that G′/G″ cross-over point during temperature ramp test may not depict the actual gelation temperature of the materials taking into account a larger range of frequencies [30,31]. Gels are usually accepted to display solid-like mechanical profiles, where the storage modulus (G′) is greater than the loss modulus (G″) throughout the evaluated frequency range at low amplitude [2], typically accompanied by a low dependence of both moduli on frequency. Viscoelastic materials may alternatively display Maxwell-type behaviour, in which G″ > G′ at low frequencies, associated with a system which doesn't exhibit dominant long-range elastic interactions, but on increasing frequency reaches G′ > G″ at a characteristic relaxation time (1/angular frequency) as a result of shear-induced elastic interactions in the system. The importance of this discrimination is that Maxwell materials may exhibit liquid-like behavior at low shear over long timescales, for example during storage in a container. Gel strength has sometimes been depicted by the ratio of the storage moduli to the loss moduli of the samples at low frequency [1], however any ratios of this type express the relative elastic versus viscous behavior and not the overall resistance to deformation.

In situ gelling drug formulations with gelation temperature above 37 °C may not be suitable for transmucosal drug delivery to humans [32] as such formulations may remain as liquid at physiological temperature; resulting in biological fluid dilution and wash off, of the drug formulations; short-lived mucosal retention of the drug formulation, and reduced therapeutic effectiveness.

Examples of temperature sensitive drug delivery systems are chitosan/beta-glycerophosphate systems [1], polyethylene oxide-polypropylene oxide-polyethylene oxide (Poloxamers) [8], and poly (N, N-diethyl acrylamide)-b-poly (ethylene glycol)-b-poly (N,N-diethyl acrylamide) [9]. The temperature-dependent gelation of chitosan/β-glycerophosphate (CHIGP) systems is shown in Fig. 2.

### 2.2. Ion-sensitive systems

Ion-responsive polymers used to formulate ion sensitive in situ gelling systems generally possess ionisable groups. Polymeric anions cross-link with some monovalent ($Na^{+}$) and/or divalent ($Mg^{2+}$ and $Ca^{2+}$) cations present in different physiological fluids, such as saliva, tears, nasal fluid, etc. They display sol–gel transition upon electrostatic interaction between oppositely charged species from the drug carrier and the biological fluid [33]. The type and concentration of cation that cross-link the ion sensitive polymers dictates the viscosity of the cross-linked polymer and the polymeric system's sol–gel transition rate [34]. In addition, changes in ionic strength and ionic composition of the target sites due to pathological conditions may facilitate sol–gel transition of the ion-sensitive dosage forms [34].

The gelation potential of ion-sensitive delivery system can be evaluated by mixing simulant biological fluid (tear fluid, nasal fluid or vaginal fluid) with the in-situ gelling samples and changes in their rheological profiles investigated using viscometry or turbidimetric analysis [35].

Examples of ion sensitive drug delivery systems are pectin, sodium alginate, gellan gum [36], and methacrylated gellan gum [10]. It is worthy of note that gellan gum, an anionic polysaccharide composed of 1,3-β-D-glucose, 1,4-β-D-glucoronic acid, 1,4-β-D-glucose and 1,4-α-L-rhamnose repeat units, exhibits temperature-dependent, pH-responsive and ion-induced gelation [37]. It transforms into gel via complexation with cations and hydrogen bonding with water, resulting in the formation of double helical junction zones and a three-dimensional gel network [33]. Clay gels have also been shown to exhibit ion sensitivity in their gelation [38].

### 2.3. pH sensitive systems

The gelation behavior of pH responsive formulations is influenced by the constituent polymer's pKa. Weakly acidic or basic pH sensitive polymeric solutions typically transform into gel at pH levels lower than their pKa values or greater than their pKa, respectively [39]. In addition, variation in the pH of the physiological environment could facilitate changes in the polymer's ionization state, conformation, and solubility, resulting in the gelation of the drug carrier [39].

Typically, pH sensitive polymers have ionizable weakly basic or weakly acidic moieties on their backbone. For example, polymers with weakly acidic groups, such as poly (methacrylic acid) containing carboxylic acid, become deprotonated at alkaline pH (above their pKa values) and acquire negative charge, resulting in increased electrostatic repulsion between polymer molecules which can in turn lead to physical transitions [33]. pHs lower than the pKa can thus promote polymer–polymer interactions, such as H-bonding between COOH pairs, which are required for gel formation. pHs above the pKa will transition the polymer to a polyelectrolyte which is likely to lead to polymer–polymer repulsion and will typically form viscous solutions, dependent upon factors including molecular weight and ionic strength of solution.

Polymers with weakly basic groups such as amines become protonated at acidic pH less than the pKa of their conjugate acid ($pK_aH$), and

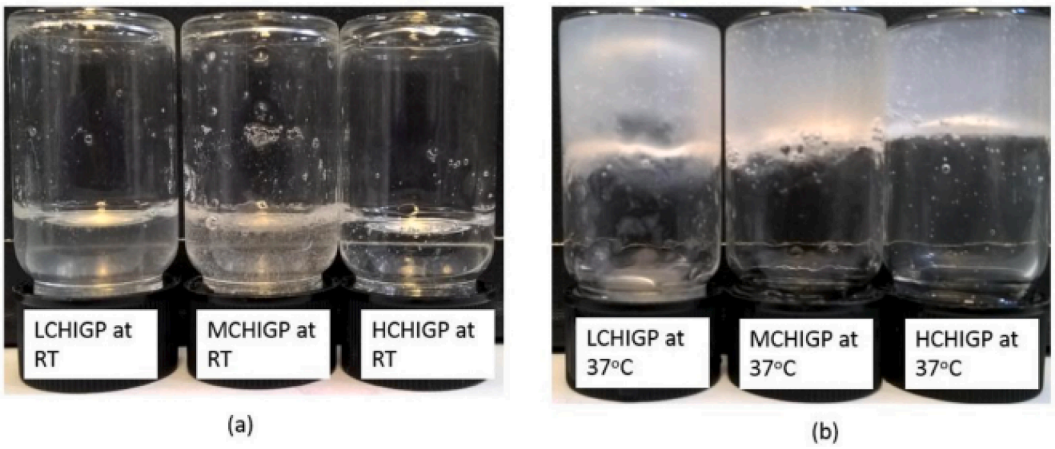


**Fig. 2.** Exemplar images of chitosan/beta-glycerophosphate formulations prepared using three chitosan grades, maintained at (a) room temperature and (b) 37 °C [1].

are typically uncharged above it, and thus display the reverse behaviour of weakly acidic systems. pH-responsive systems may alternatively be designed by exploiting chemical reactions which are favoured/disfavoured dependent upon pH and which lead to covalent interaction between chains [40].

Examples of pH sensitive formulations are polyacrylic acid-based in situ nasal gels (Carbopol 934) that exhibit in situ gelling properties by deprotonation at nasal pH ≈ 8.3 in patients with rhinitis [41]; polyacrylic acid based formulations for colonic delivery of 5-aminosalicylic acid [42]; and xanthan-based systems used for controlled bovine serum albumin release at physiological pH [43].

## 3. Bioactive agent containing in situ gelling delivery studies

Various in situ gelling formulations have been delivered to various body sites via the buccal, ocular, nasal, vaginal, cervical, intravesical, and intraperitoneal routes of drug administration. The rheological properties and clinical findings from studies on in situ gelling systems over the last decade are detailed on Table 1, to be detailed further in the subsequent sections of the manuscript.

### 3.1. Buccal formulations

Mucoadhesive in situ gelling delivery systems have been used to deliver therapeutic agents to the buccal mucosa within the oral cavity in order to alleviate discomfort associated with oral mucositis and esophagitis, common amongst head and neck cancer patients undergoing chemotherapy and/or radiation treatment [6].

Buccal dosage forms have been formulated using K-carrageenan, which is a thermosensitive and ion-responsive copolymer of sulfated ester of galactose and 3,6-anhydrogalactose, with one negative charge per disaccharide residue, that exhibits anti-oxidant and anti-inflammatory properties [44]. It transforms into gel at elevated temperatures and in the presence of ions such as $K^+$, $Na^+$, $Mg^{2+}$ and $Ca^{2+}$ via transition from its coil to helix conformation, followed by helix aggregation.

Vigani and coworkers prepared bioactive Hibiscus sabdariffa extract (HSE; 0.2 %)-containing calcium chloride (0.04 %), k-carrageenan (0.4 % or 0.6 %)/hydroxypropyl cellulose (HPC; 1 %)-based in situ gelling systems and studied their rheological properties using rheometer [44]. K-carrageenan exhibited wound healing properties and enabled the formulation to transform into gel in presence of saliva ions and calcium chloride, while HPC enhanced the mucoadhesive properties of the formulations [44].

Normalised rheological synergism parameters obtained for 0.4 % HSE and 0.6 % HSE containing formulations were 1.44 ± 0.9 and 1.19 ± 0.06, respectively, suggesting that both formulations exhibited comparable degree of interaction with saliva ions. Also, the gelling capacity of the formulation was strengthened by loading increased concentration of the bioactive extract, in terms of their reduced loss tangent values compared to the blank formulations [44]. The Hibiscus extract in the formulation improved its compatibility with human dermal fibroblasts and capability to promote cell proliferation. HSE and HSE-containing formulations exhibited comparable inflammatory properties in terms of their interleukin-8 release after cell treatment [44]. These findings revealed that the in-situ gel formulation containing 0.6 % HSE may be preferred for the potential treatment of oral mucositis due to its rheological and biological properties.

Periodontitis is an immune-inflammatory condition of the periodontal tissues that starts off as microbial tooth infection and degenerates into destruction of the sub-gingival tissues, periodontal ligament and the alveolar bone, resulting in gingival recession and creation of periodontal pockets that supports growth of anaerobic Gram-negative bacteria. Kassem and coworkers have incorporated meloxicam (anti-inflammatory agent) or minocycline hydrochloride (antimicrobial agent) into poloxamer (35 % w/v) based thermosensitive gels or carbopol 1 %/HPMC 2.5 %-based pH sensitive gel, for potential treatment of periodontitis [45]. Poloxamer gels were preferred as the formulation did not compromise the stability of meloxicam and minocycline HCl.

The poloxamer based formulation facilitated release of 21.72 % of meloxicam over a week and 85 % of minocycline HCl over three days. Also, the drug carrier reduced periodontal pocket depth, gingival inflammation and alveolar bone density of periodontitis patients [45]. However, the authors did not state the viscosity or viscoelasticity values of the formulations. Nevertheless, visual examination of the drug formulations revealed that Carbopol/HPMC gel systems were not suitable for the delivery of minocycline hydrochloride as the formulation liquefied and drug precipitated, probably due to the positive charge and acidic nature of minocycline, which may disturb the electrostatic repulsion between adjacent ionized carboxyl groups, lowering the pH of the drug formulation, decreasing polymer ionization and preventing polymer gelation [45].

Recently, composite system of moxifloxacin HCl loaded PLGA/PVA based nanoparticles and poloxamer-based thermosensitive gel were studied for periodontitis treatment [46]. The optimised drug loaded nanoparticle exhibited particle size of 288 nm and satisfactory drug entrapment (81 %) and biphasic drug release profile, while the composite system of the most promising nanoparticles and in situ gel displayed gelation temperature of 37 °C, gel strength of 108 g, and bioadhesive strength of 12 g, which is required for improved retention of

**Table 1**
In situ gelling formulations classified based on their route of administration.

| Route | Therapeutic agent/Delivery system | Clinical findings | Potential Indications | Ref |
|---|---|---|---|---|
| **Buccal** | Hibiscus sabdariffa extract-containing k-carrageenan(k-CG)/hydroxypropyl (HPC)-based thermo- and ion-sensitive in situ gelling systems | Hibiscus sabdariffa extract-containing formulations exhibited satisfactory gelation potential and elasticity based on their rheological synergism and loss tangent values; the presence of the bioactive extract did not significantly affect the mucoadhesiveness of the formulation. | Oral mucositis and esophagitis treatment | [44] |
| | Meloxicam or minocycline hydrochloride-containing poloxamer based thermosensitive gels or Carbopol/HPMC based pH sensitive gels | Poloxamer based gels were more promising than carbopol/HPMC based formulations as it did not compromise the stability of minocycline. It also facilitated the release of meloxicam (21.72 %) within a week as well as 85 % of minocycline HCl over three days. Treated periodontitis patients experienced improved clinical outcomes. | Local intra-pocket periodontitis treatment | [45] |
| | Moxifloxacin HCl loaded PLGA nanoparticles/poloxamer-based thermosensitive gel | The optimised formulation displayed faster ease of gelation and gel strength required for improved retention in the periodontium. In vivo histopathological studies of the periodontal tissues of treated patients revealed that the new drug carrier was more biocompatible than the marketed moxifloxacin product. | | [46] |
| **Ocular** | Pilocarpine loaded gelatin-graft-poly(N-isopropylacrylamide) gels | The LCST of gelatin-PNIPAAm copolymer was dependent on the number-average molecular weight of the thermoresponsive polymer. Also, Mercaptoacetic acid/N-isopropylacrylamide ratio influenced their degradation and controlled pilocarpine release profile. | Glaucoma treatment | [7] |
| | Pilocarpine loaded chitosan-graft-poly(N-isopropylacrylamide) thermosensitive gels | The ratio of chitosan to poly (N-isopropylacrylamide modulated the LCST of the resultant copolymeric hydrogels. Prolonged antiglaucoma activity for 42 days was evident using a rabbit model | | [47] |
| | Brinzolamide/ Amberlite IRP-69 ion exchange resin loaded Poloxamer F127/Carbopol 934P based thermosensitive gels | The stable, non-irritant formulation exhibited gelation temperature of 33.2 ± 1.1 °C with pseudoplastic flow behavior. Brinzolamide (80 %) was released from the new formulation over 8 h; remarkable increase in drug bioavailability was evident within the aqueous humor compared to commercial brinzolamide eye drops. | | [48] |
| | Betaxolol hydrochloride loaded P407/P188/ polycarbophil based thermosensitive gels | Optimised stable formulation exhibited viscosity of 243.3 ± 5.80 mPas at 5 $s^{-1}$; respective GT of 25.6 °C and 34.6 ± 0.15 °C before and after simulant tear fluid dilution; prolonged drug release for 8 h; enhanced drug bioavailability; and reduced intraocular pressure. | | [11] |
| | Brinzolamide containing gellan gum-based ion-sensitive gels | Viscosity of the formulation increased from 19 cPs at 25 °C to 90 cPs at 34 °C while the gelation time of formulation/simulant tear fluid (pH 7.4) mixture (1:20) at 34 °C decreased from 18 s to 3 s as the concentration of gellan gum was increased from 0.1 % w/v to 0.5 % w/v. Optimised formulations containing 0.25 % w/v GG exhibited controlled drug release in vitro, and the duration of IOP reduction was prolonged, in comparison to Azopt® (ocular residence time: 17.7 h versus 4.9 h) | | [49] |
| | Pilocarpine containing gellan gum and methacrylated gellan gum-based ion-sensitive gels | Fluorescein sodium loaded GG, LMeGG, MMeGG and HMeGG exhibited simulant tear fluid wash $out_{50}$ profile of 18 mL, 25 mL, 65 mL and 75 mL, respectively, inferring that the bovine conjunctiva mucoadhesiveness of the in-situ gels was enhanced with increased degree of methacrylation of gellan gum. | | [10] |
| | Dexamethasone nanoparticles loaded P407/P188-based thermosensitive gels | Thermosensitive gel and gel-nanoparticulate samples exhibited a gelation temperature of 32.7 °C and 34.3 °C, respectively. They exhibited controlled drug release, improved corneal retention/permeability; and in vivo rabbit studies on composite nanoparticulate gel revealed superior efficacy relative to commercial TobraDex eye drops | Ocular anti-inflammatory activity | [50] |
| **Nasal** | Composite system of dexamethasone loaded lipid/ alginate nanoparticles and pectin in situ gel | The loss and storage modulus of pectin gel was slightly lower than that of drug nanoparticulate gel samples. The nanoparticulate gel exhibited sustained drug release profile in comparison to dexamethasone nanoparticles (t50%: 2.1 h versus 1.7 h) | Chronic rhinosinusitis treatment | [51] |
| | Sprayable Fluticasone loaded pectin/gellan gum/sodium hyaluronate/Tween 80-based in situ gelling system | Optimised formulation exhibited greater suspension stability, narrower spray cone angle, higher turbinate deposition to posterior regions of nasal cavity | Allergic and non-allergic rhinitis, chronic rhinosinusitis and nasal polyposis. | [52] |
| | Tranexamic acid containing Chitosan/beta-glycerophosphate (1.4%/18.8 %)-based nasal spray | The new formulation exhibited gelation temperature and time of 33 °C and 5 min, respectively. It was non-irritant to human nasal epithelium and demonstrated six times | Nasal wound treatment | [53] |

(*continued on next page*)

**Table 1** (*continued*)

| Route | Therapeutic agent/Delivery system | Clinical findings | Potential Indications | Ref |
|---|---|---|---|---|
| | | faster wound closure compared to the control tranexamic solution. | | |
| **Vaginal** | Metronidazole containing Pluronic F127/F68 (20 %/10%)-based thermosensitive gels | Optimised Pluronic based in situ gel was biocompatible, exhibited gelation temperature of 28 °C and viscosity of 2.22 × 105 mPas at 37 °C, and exhibited mucodhesive and sustained drug release profile, in comparison to marketed metronidazole gel | Bacterial vaginosis treatment | [54] |
| | Amoxicillin trihydrate containing P407/chitosan lactate (CHILP407) and glycerophosphate/chitosan lactate (CHILGP) systems | CHILGP with or without simulant vaginal fluid exhibited greater storage and loss moduli values than CHIP407 based in situ gels at 37 °C. Also, CHILGP based formulations exhibited greater mucoadhesiveness, antimicrobial and wound healing potential than P407-based formulation | Vaginal mucositis treatment | [55] |
| | *Lactobacillus gasseri* containing P407/MC/Pectin/ xyloglucan-based thermosensitive gels | The optimized formulations were stable, mucoadhesive, biocompatible and retained gelation potential in the presence of simulant vaginal fluid, based on their viscosity values at 37 °C under shear rate of 5 $s^{-1}$ | Prevention of vulvovaginal candidiasis recurrence | [56] |
| | 0.3 M sodium chloride/ Tenofovir disoproxil and progesterone containing PDEA-b-PEG-b-PDEA thermosensitive gel | The optimized formulation was biocompatible and stable for at least 12 weeks. It exhibited gelation temperature of 36 °C, gelation time of 67 s, and a yield stress of 862 Pa, which is desirable for bioprinting | HIV prophylaxis and luteal phase support | [9] |
| **Cervical** | Composite system of paclitaxel loaded MPEG-PCL polymeric micelles and cisplatin containing thermosensitive PEG-PCL-PEG gel | The composite system exhibited gelation temperature<30 °C. In vivo studies revealed that the composite system of hydrogel and nanoparticles exhibited improved tumour growth inhibition, prolonged mice survival time, decreased CD31, CD133 and ALDH1 expression, G1 phase arrest and apoptosis induction compared to cisplatin hydrogels or paclitaxel nanoparticles | Cervical cancer treatment | [57] |
| | Composite system of toad venom (TV) loaded solid lipid nanoparticles and nanorealgar containing poloxamer 188/407 (F127/F188)-based thermosensitive in situ gel | The optimized formulation was stable, biocompatible and displayed a gelation temperature of 33 ± 0.9 °C. It also significantly improved anti-cervical and -ovarian cancer effects in vitro. | | [58] |
| **Intravesical** | Mitomycin-C containing chitosan/beta-glycerophosphate based thermosensitive systems | LCHIGP, MCHIGP and HCHIGP exhibited gelation temperature of 30.4 ± 0.3 °C, 29.8 ± 0.2 °C. and 29.6 ± 0.1 °C, respectively. Also, they displayed comparable gelation time based on rheology (1.6 min versus 1.4 min versus 1.0 min). Chitosan molecular weight influenced their gelation, mucoadhesive and drug release profile. HCHIGP was the most promising formulation for potential intravesical treatment of bladder cancer | Bladder cancer treatment | [1] |
| | Doxorubicin-loaded composite system of human serum albumin (HSA) nanoparticles and poloxamer 407 thermosensitive gel | NP-Dox-Gel exhibited GT of 10 °C and Gt of 2 min while doxorubicin loaded nanoparticles and non-floating hydrogel displayed GT of 12 to 18 °C and Gt of 2–5 min. | | [59] |
| | Deguelin loaded DOTAP and monomethoxyl poly (ethylene glycol)-poly ε-caprolactone hybrid nanoparticles/Pluronic F127 hydrogels | Fluorescent composite drug carrier was syringeable at 25 °C and gel was observed in the mice's bladder within 10 min of intravesical administration and residence of the D/DMP-F gel was sustained for 2 h. | | [60] |
| | Composite system of chitosan-thioglycolic acid conjugate (CHI-TGA) nanoparticles (CHI-TGA NPs) and 2 % chitosan gel (CHI-TGA NPs/CHI) or in situ poloxamer gel (CHI-TGA NPs/Plx gel) | The poloxamer based composite nanoparticulate hydrogel system was more syringeable than the chitosan-based formulations based on the work done to expel syringe content: CHI-TGA NPs/Plx gel (30.7 ± 1.4 Nmm) versus CHI-TGA NPs/CH gel (82.8 ± 0.8 Nmm). The amount of drug released after 4 h from CHI-TGA NPs was greater than that of CHI-TGA NPs-CHI gel and CHI-TGA NPs-Plx gel (51.0 ± 3.7 % versus 33.4 % ± 5.0 % versus 19.6 % ±1.6 %). | | [61] |
| | Composite system of rapamycin loaded folate modified liposomes and poloxamer hydrogels | All the studied liposomal gel formulation exhibited similar gelation temperature and gelation time of 21 °C and 29 s, respectively. Composite system of rapamycin loaded folate modified liposomes and poloxamer gel exhibited superior antitumor activity relative to other studied formulations | | [62] |

the drug formulation in the periodontium. Incorporation of nanoparticles into gel reduced burst drug release effect. Based on in vivo histopathological studies, treatment of diseased periodontal tissues for one week using the new composite system of in situ gel and moxifloxacin nanoparticles was more effective than those tissues treated for three weeks using marketed moxifloxacin containing gel formulation [46].

### 3.2. Ocular formulations

The majority of reported ophthalmic in situ gelling formulations are targeted towards the eradication of glaucoma, which is an ocular disorder that could result in the irreversible loss of vision and blindness [6]. This pathological condition is typically managed by frequent drug administration, surgery and laser surgery [6]. The main classes of drugs used to manage glaucoma are beta-blockers (betaxolol, timolol), cholinergic agonists (pilocarpine), α2-agonists (brimonidine), carbonic

anhydrase inhibitors (brinzolamide), etc. The carbonic anhydrase inhibitors are indicated for patients that are unresponsive to beta-blockers or they have medical conditions for which their use is contraindicated [49].

Liquid formulations that are topically applied to the eyes transform into gel when they become deposited within the conjunctival cul-de-sac, facilitating controlled drug release; prolonged therapeutic effect; reduced dosing frequency, thereby improving patient compliance [11].

Pilocarpine was incorporated into gelatin-graft-poly(N-isopropylacrylamide) (PNIPAAm) thermosensitive gel to improve drug ocular residence time and ocular bioavailability [7]. The rheological property of the drug formulation was studied using differential scanning calorimetry (DSC) and the onset of the endothermic peak depicted the lower critical solution temperature of the formulation [7]. The study was carried out in a nitrogen environment and the temperature of the graft copolymeric formulation-containing pan was increased from 25 °C to 45 °C at the rate of 3 °C/min and the rheological behaviour of the drug formulation was evaluated.

The three studied gelatin/PNIPAAm graft-copolymeric formulations differed in terms of their mercaptoacetic acid (MAA)/N-isopropylacrylamide (NIPAAm) molar ratio, with G-M/N005, G-M/N025 and G-M/N125 depicting samples with MAA/NIPAAm ratio of 1: 0.05, 1:0.25 and 1:1.25, respectively. G-M/N005 (MW 11.08 ± 0.35 kDa), G-M/N025 (MW 4.44 ± 0.27 kDa), and G-M/N125 (MW 2.59 ± 0.32 kDa) exhibited lower critical solution temperature of 31.4 ± 0.2 °C, 32.7 ± 0.1 °C, and 33.0 ± 0.1 °C, respectively, inferring that the temperature of gelation of the graft copolymeric system improved with increased molecular weight of the polymer used to prepare in situ gel formulation [7].

The amount of drug released after 14 days of study in balanced salt solution medium at 34 °C from G-M/N005 was 36.0 μg/mL while that of G-M/N025 and G-M/N125 was approximately 5 μg/mL. All the studied formulations were biocompatible based on their BCE CID-Ib cytotoxicity testing. Various GN-based pilocarpine carriers displayed unique IOP lowering effects and mitotic activity [7], suggesting that the MAA/NIPAAm molar ratio of in situ gelling systems could modulate the physical and biological properties of new antiglaucoma formulations.

In a latter study, the gelatin segment was replaced with chitosan (chitosan-g-poly(N-isopropylacrylamide) (CHI-PNIPAAm) in order to prolong the duration of release of pilocarpine as well as improve ocular drug bioavailability [47]. CHI-PNIPAAm 10 and CHI-PNIPAAm 20 were prepared using 10 g and 20 g of carboxylic end-capped PNIPAAm, respectively, The LCST of aqueous solution of thermosensitive samples (10 % w/v) were also studied using DSC under similar experimental conditions reported by Lai in 2013 [7]. The respective LCST for CHI-PNIPAAm 10 and CHI-PNIPAAm 20 was 31.9 ± 0.2 °C, and 29.5 ± 0.3 °C, suggesting that the number of PNIPAAm chains grafted onto chitosan influenced their temperatureof gelation [47]. Based on WST-1 assay, the biocompatibility of the studied formulations was independent of the number of PNIPAAm chains grafted to chitosan. The amount of thermo-responsive polymer segments grafted onto the chitosan influenced the phase transition temperature and enzymatic degradability of Chi-PNIPAAm-based drug carriers [47]. The delayed degradation of Chi-PNIPAAm 20 system induced sustained pilocarpine release and facilitated the release of therapeutic amount of pilocarpine over 42 days and reduction in intraocular pressure evident in treated glaucomatous rabbits (Fig. 3). These findings suggested that the new formulations would be effective for the treatment of glaucoma [47].

The clinical use of commercially available brinzolamide suspension formulations (Azopt®) intended for glaucoma treatment is limited by its high cost, poor residence within the precorneal space after instillation, which results in poor intraocular bioavailability (10 %), necessitating frequent dosing of drug formulation and poor patient compliance [49].

Li and coworkers incorporated brinzolamide into ion exchange resin and loaded the drug resin into Poloxamer F127/Carbopol 934P-based mucoadhesive, thermosensitive gels for improved drug retention within the aqueous humour of the eyes [48]. Apart from using DSC to

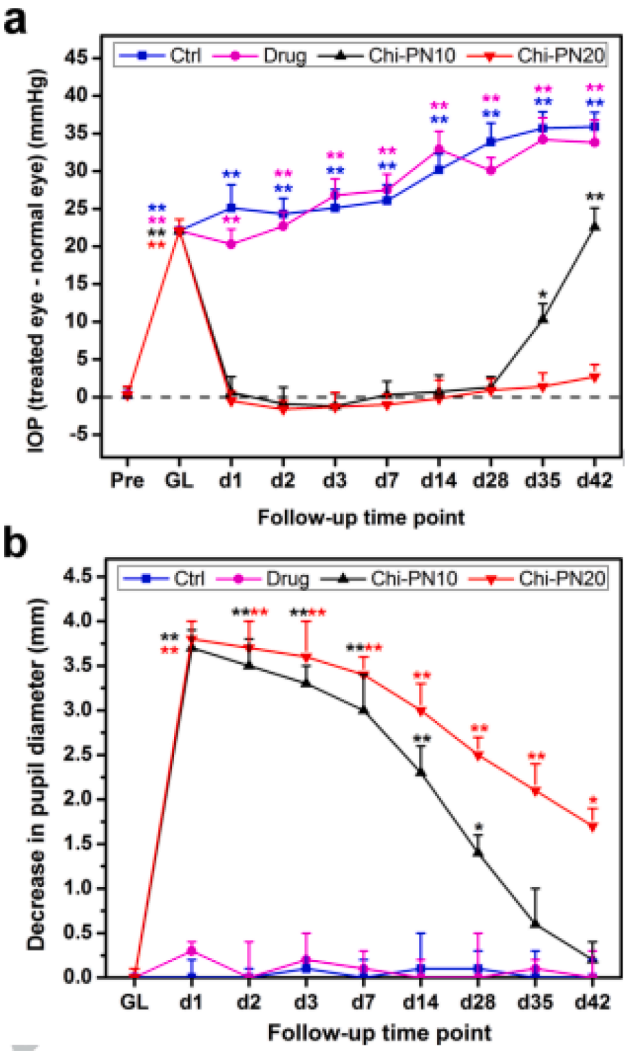


**Fig. 3.** (a) Intraocular pressure and (b) pupil diameter of rabbits with glaucoma after intracameral injection of free pilocarpine solutions and various pilocarpine containing chitosan-g- PNIPAAm samples. Glaucomatous animals receiving no treatment served as the control groups. Asterisks indicate statistically significant differences (*p < 0.05, ** p < 0.005; n = 6) as compared with the baseline, IOP and pupil diameter values. Follow-up time point: pre-operation (Pre), day (d), reproduced with permission [47].

study their gelation temperature, the gelation time of the drug formulation with or without simulant tear fluid was evaluated in terms of the length of time required for magnetic stir bar mixing the in-situ gelling formulation to stop moving and sample flow ceased.

In addition, viscometry was used to study the viscosity of the samples at 25 °C and 35 °C under various shear rates (6–60 rpm) in order to evaluate the effect of temperature elevation and shear stress on the rheological properties of the drug formulation. The optimized formulation contained 22 % of poloxamer, and the in situ gelling tendency of the formulation was not significantly compromised with simulant tear fluid (STF) dilution [48]. Brinzolamide (80 %) was released into artificial tears from the new formulation and conventional eye drops over 8 h and 2 h, respectively, indicating that the new drug product exhibited sustained drug release profile [48].

Bhalerao and coworkers investigated the in-situ gelling potential of brinzolamide-containing gellan gum-based ion sensitive formulations using viscometry and vial inversion method [49]. The intrinsic viscosity of the formulation at 25 °C and 35 °C was 19 cPs and 90 cPs, respectively while the gelation time of formulation/simulant tear fluid (pH 7.4, 34 °C) mixture (1:20) decreased from 18 s to 3 s as the concentration of

gellan gum was increased from 0.1 to 0.5 % w/v under shear stress of 50 rpm [49]. However, formulations containing 0.4–0.5 % may not be suitable for ocular administration due to their highly viscous nature. Optimised formulation containing 0.25 % w/v of gellan gum reduced intraocular pressure from 25 to 28 mmHg to 12–14 mmHg after instillation to rabbit eyes. Also, the ophthalmic gel exhibited longer ocular residence than commercial brinzolamide suspensions (Azopt®, 1 % w/v) (7.4–17.7 h versus 4.9 h) [49]. However, the dimethyl sulfoxide containing gellan gum-based formulation may pose long term ocular irritant potential, limiting its clinical translation and patient acceptability.

The composition of betaxolol hydrochloride containing P407/P188/polycarbophil (PCP) based thermosensitive gels studied by Huang and coworkers was P407 (22 % w/v), P188 (3.5 %), and PCP (0.2 %). The in situ gelling capacity of the formulations before and after simulant tear fluid dilution were studied using a viscometer [11], and the viscosity of the formulations was determined at a shear rate of 5 $s^{-1}$ (300 rpm), which may not depict the intrinsic viscosity of the samples. The gelation temperature of the optimised formulation with and without STF exhibited gelation temperatures of approximately 34 °C and 26 °C, respectively. Incorporation of polycarbophil into poloxamer based in situ gelling systems did not compromise their gelation potential and rheological properties. The amount of drug released from formulations was approximately 100 % the drug loaded into betaxolol solution, commercial betaxolol and betaxolol containing in situ gelling formulation, released over 2 h, 3 h and 8 h, respectively, indicating that the new formulation displayed a sustained drug release profile [11]. The intraocular pressure-lowering effect of the new formulation on glaucomatous rabbits treated for 7 days was greater than that of the commercial drug suspension. Overall, the new formulation was stable, non-irritant and exhibited improved ocular bioavailability, suggesting that the new betaxolol containing poloxamer/carbophil based dosage form will be beneficial to manage glaucoma.

Agibayeva and coworkers synthesised three types of methacrylated gellan gum (LMeGG, MMeGG, and HMeGG) that differed in terms of their degree of methacrylation, and their physicochemical and biological properties compared with that of unmodified gellan gum. They used dynamic light scattering to evaluate the degree of aggregation of ion-sensitive gellan gum-based ophthalmic in situ gels. Highly polydisperse aggregates, characteristic of colloidal dispersions, were evident in both gellan gum and its methacrylated analogues [10]. Their aggregation potential was increased with methacrylation of the gellan gum due to the relatively hydrophobic behaviour of methacryloyl groups. The extent of aggregation of all the studied drug carriers was further enhanced with increased polymer concentrations from 0.1 to 1 mg/mL and also under highly acidic pH (pH 2.0), probably due to suppression of carboxylic groups ionization. The mucoadhesive properties of the studied formulations were evaluated as a function of the volume of simulant tear fluid (STF) that washes off 50 % of the florescent samples instilled on animal mucosal surfaces (STF $WO_{50}$), quantified using fluorescence microscopy (Fig. 4).

The STF $WO_{50}$ profile of gellan gum, LMeGG, MMeGG, and HMeGG was 18 mL, 25 mL, 65 mL and 75 mL, respectively, inferring that the ion-sensitive solutions transformed into gel in the presence of simulant tear

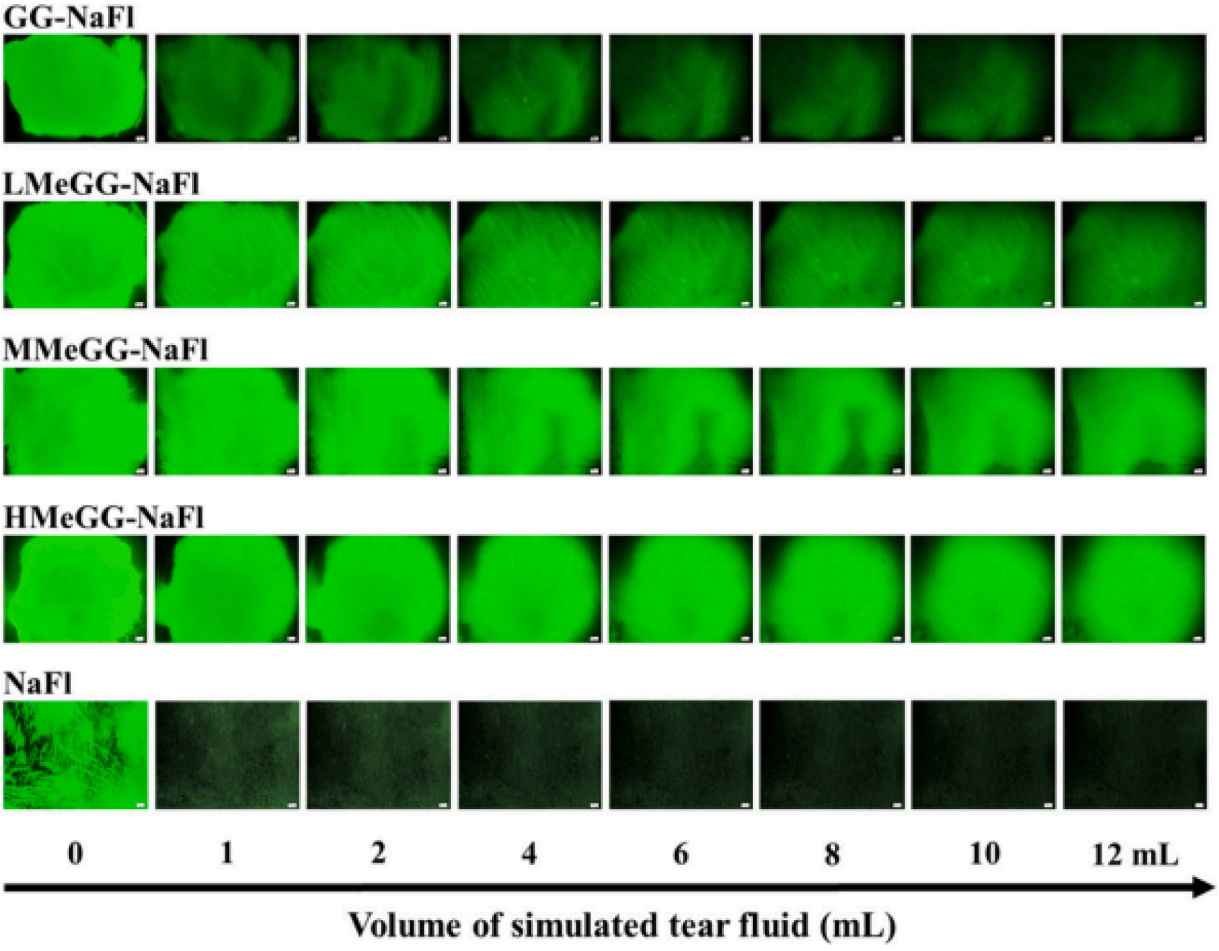


**Fig. 4.** Exemplary fluorescent microphotographs depicting mucoadhesiveness of unmodified and methacrylated gellan gum formulations (GG, LMeGG, MMeGG and HMeGG) on freshly excised bovine conjunctival tissue washed with simulant tear fluid (pH 7.4; 200 μL/min) over 1 h, scale bars are 200 μm; fluorescein sodium served as the control [10], reproduced with permission.

fluid, and their mucoadhesiveness was enhanced with increased extent of methacrylation [10]. Surprisingly, in vivo rabbit studies revealed that conjunctiva drug retention was greatest with pilocarpine hydrochloride containing LMeGG-based in situ gel formulation [10].

Infections, inflammation and traumatic corneal disorders are responsible for corneal neovascularization, resulting in diminished corneal clarity and visual impairment [63]. Recently, drug nanoparticles were incorporated into in situ gels due to their potential to prolong drug release, thereby reducing dosing frequency and improving therapeutic outcomes of patients with various diseases. Dexamethasone (DXM) is an anti-inflammatory synthetic glucocorticoid that is commonly used in the treatment of ocular inflammation following cataract surgery or corneal procedures [50].

Wen and coworkers prepared dexamethasone nanoparticles containing P407 (18 % w/w)/P188 (2 % w/w)-based thermosensitive gels in order to improve the aqueous solubility and ocular bioavailability of the drug [50]. The optimized formulations were studied using rheometry and tube inversion method. The in situ gel and gel-nanoparticulate formulations exhibited a gelation temperature of 32.7 °C and 34.3 °C, respectively [50], indicating that the presence of nanoparticles in the gel formulations did not dramatically alter gelation. In addition, their gelation temperatures were below the physiological temperature (37 °C). There was a 4.84 and 5.22 fold-increase in the corneal permeability of in situ gel and NPs-gel, respectively, in comparison to that of TobraDex, a commercial dexamethasone containing ocular formulation [50]. The NPs-gel displayed the greatest corneal permeation, revealing that it can serve as a drug reservoir to sustain dexamethasone for prolonged period of time [50]. All the studied formulations displayed ocular biocompatibility (Fig. 5).

### 3.3. Nasal formulations

The nasal route has been used to deliver a wide range of small compounds and biological macromolecules such as peptides, proteins and vaccines. Also, common diseases treated using nasal dosage forms include allergic and infectious rhinitis, sinusitis, nasal epithelial lesions, and rhino-sinusitis [64]. In addition, therapeutics could be delivered to the brain through the nose due to the highly vascularised nature of the nasal mucosa that permits brain drug delivery via the olfactory neuroepithelium [6].

Composite system of dexamethasone loaded lipid/alginate nanoparticles and pectin based in situ gels have been studied for chronic rhinosinusitis therapy [51]. In situ gel was formed in the presence of calcium ions in the nasal mucosa. Rheometry was then used to evaluate the viscoelastic properties of the formulations. The rheological behavior of pectin gel with or without dexamethasone nanoparticles were comparable, though the loss and storage moduli of pectin gel was slightly lower than that of drug nanoparticulate gel samples, probably due to electrostatic repulsion between negatively charged pectin and alginate, decreasing viscoelasticity of the composite delivery system [51]. Drug release profile was evaluated as a function of the length of time for 50 % of the loaded drug to be released (t50%). The dexamethasone nanoparticulate gel formulation facilitated superior controlled drug release relative to the drug nanoparticles and free drug (t50%: 2.1 h versus 1.7 h versus 0.6 h) [51].

Sprayable fluticasone loaded sodium hyaluronate/pectin/gellan

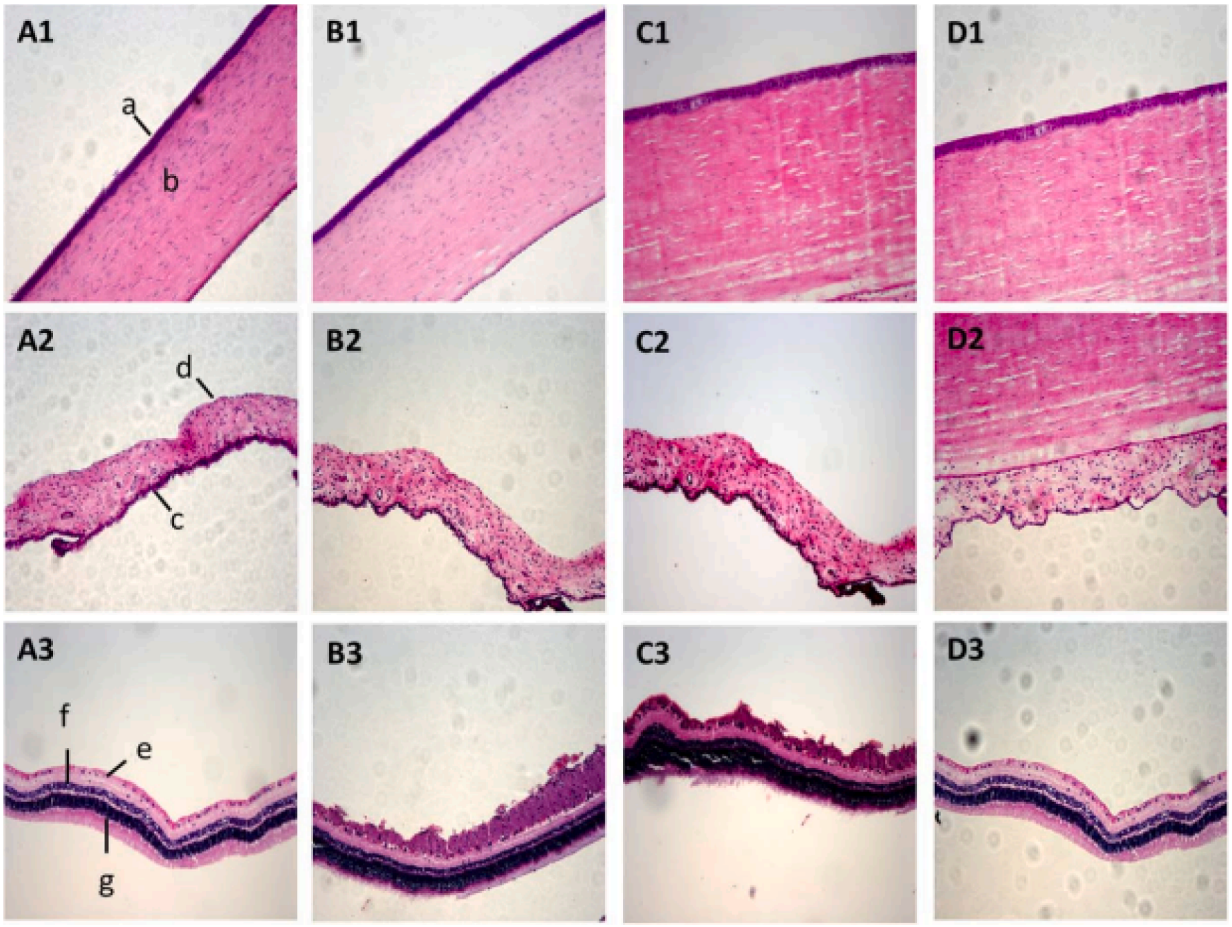


**Fig. 5.** Histological images of the cornea (1), iris (2), and retina (3) after treatment with: a control (A), tobramycin dexamethasone eye drops (B), an in situ gel (C), and nanoparticle-containing in situ gel (D) for 7 days (n = 3, magnification 20 x) and tissue structure showing the epithelial layer (a), stromal layer (b), posterior limiting layer (c), anterior limiting layer (d), ganglional cell layer (e), inner nuclear layer (f), outer nuclear layer (g). Reproduced with permission [11].

gum based in situ gelling systems were developed by Nizic and coworkers, with sodium hyaluronate serving as the gel structuring and bioactive component while Tween 80 served as the suspending agent [52]. Rheometry was used to study the viscosity and gelation potential of the formulations. Satisfactory weak gels were generated immediately after mixing formulation and simulant nasal fluid (1:1), with storage modulus greater than loss modulus throughout the study period. The optimised formulation contained 0.05 % fluticasone, 0.03 % Tween 80, 0.7 % pectin, 0.05 % sodium hyaluronate administered at 45° from the horizontal plane at an inspiratory flow of 30 L/min. It exhibited zero-shear viscosity of 11.16 ± 0.10 mPas, revealing greater drug suspension stability, narrower spray cone angle, higher turbinate deposition, and reduced possibility of premature drug deposition to the anterior region of the nasal cavity [52].

Gholizadeh and coworkers reported the use of sprayable tranexamic acid containing a chitosan/beta-glycerophosphate-based nasal formulation for the treatment of nasal wounds [53]. Water bath-based inverted tube technique and rheology were used to evaluate the gelation tendency and viscoelastic properties of the formulation. The optimized formulation containing chitosan 2 % w/v, beta-glycerophosphate 49 % w/v at a volume ratio of 4: 2.5 exhibited gelation time and temperature of 5 min and 33 °C, respectively, indicating that the formulation was suitable for nasal drug delivery. An increase in drug concentration from 0.1 % w/v to 1 % w/v induced faster gelation. Incubation of the optimized formulation at 37 °C resulted in an increased in the storage modulus values from 0.03 Pas to 168 Pas [53]. The thermosensitive formulation was biocompatible based on human nasal epithelial cell cytotoxicity testing and it facilitated an efficient wound closure treatment within 3 h, which was approximately six times faster than the control tranexamic acid solution, which is a desirable clinical property for drug delivery systems intended for epistaxis therapy [53].

### 3.4. Vaginal formulations

Antimicrobials, hormones, spermicides, anti-inflammatory and anticancer agents are major classes of therapeutic agents that are incorporated into in situ gelling formulations delivered through the vaginal route, for local and systemic effect [23]. Ibrahim et al studied the gelation potential of thermosensitive samples using viscometry and heated magnetic stirrer techniques [54]. They reported that metronidazole containing Pluronic F127/F68 (20 %/10%)- based thermosensitive in situ gel was biocompatible, exhibited gelation temperature of 28 °C; viscosity of 2.22 × 105 mPas at 37 °C; and mean mucoadhesive force of 21.2 ± 1.4 × $10^2$ ($Ncm^{-2}$)[a] [54]. The respective amount of metronidazole released from the optimized and marketed metronidazole gel formulation within 6 h was 76 % and 91 %, indicating that the new dosage form exerted a sustained drug release profile. In addition, the bacterial vaginosis cure rate after one week treatment using the new and marketed formulation was 85 % and 71 %, respectively, suggesting that the new drug product may improve the therapeutic outcomes of bacterial vaginosis patients [54].

Rossi and coworkers investigated the use of amoxicillin trihydrate containing P407/chitosan lactate (CHIL 1.6 %/P407 15 %) and glycerophosphate/chitosan lactate (CHIL 6 %/GP 8 %), for potential treatment of vaginal mucositis [55]. The rheological properties of the formulations were then studied. The addition of simulant vaginal fluid to CHILGP did not affect its gelation temperature, which remained within physiological temperature but its storage modulus values decreased in the presence of simulant vaginal fluid. On the other hand, CHILP407 displayed decreased ease of gelation with incorporation of simulant vaginal fluid. In addition, CHILGP formulations exhibited superior mucoadhesiveness, antimicrobial and wound healing potential relative to P407-based formulation [55].

Lactobacilli are probiotics that generate hydrogen peroxide, biosurfactants, antimicrobial bacteriocins, and organic acids (lactic and formic acids) that lowers intra-vaginal pH, required to inhibit growth of pathogenic organisms [56]. *Lactobacillus gasseri* containing poloxamer 407 (P407)/methyl cellulose/pectin/xyloglucan-based thermosensitive gels was studied for the prevention of vulvovaginal candidiasis recurrence [56]. Methyl cellulose (1.5 % w/w) and P407 (15 % w/w) displayed thermosensitive properties while pectin (0.5 % w/w) and xyloglucan (0.25 % w/w) served as the mucoadhesive polymers. P407 and MC improved the ease of gelation of the formulation at 37 °C alone and after dilution with simulated vaginal fluid (SVF). Pectin helped to impart appropriate pH to the formulation; exhibited low viscosity and improved their syringeability at 25 °C. A low concentration of xyloglucan (0.25 % w/w) effectively increased the mucoadhesive properties and in situ gelation potential of the formulation after dilution with SVF. The optimized formulations with or without xyloglucan exhibited satisfactory rheological and gelation potential; preserved *L. gasseri* viability; biocompatible based on HeLa cell cytotoxicity testing and stability for up to 3 weeks at 4 °C [56].

Recently, tenofovir disoproxil and progesterone containing 0.3 M sodium chloride/PDEA (20 kDa)-b-PEG (10 kDa)-b-PDEA (20 kDa) thermoreversible gels have been evaluated for intravaginal delivery of drugs for HIV pre-exposure prevention and luteal phase support [9]. Sodium chloride was added to the formulation in order to lower the intrinsic gelation temperature of the triblock copolymer (46 °C). Rheometry was used to measure the elastic properties of 30 % w/w sample, with the formulation exhibiting increased viscosity values as the temperature of the sample was increased from 20 °C to 60 °C. The optimized formulation exhibited gelation temperature of 36 °C, gelation time of 67 s, yield stress of 862 Pa, and stable at ≤ 25 °C for 12 weeks study period, which are desirable properties of topically-applied materials, which should have the ability to withstand stress [9].

SAXS data revealed that the PDEA-PEG-PDEA copolymer self-assemble into larger ellipsoidal structures due to association of the relatively hydrophobic PDEA chains at the lower critical solution temperature, resulting in increased viscoelasticity of the triblock copolymer. Hydrophobic progesterone (100 %) was released from the formulation within 32 h at 25 °C while similar amount of the biological agent was released within 144 h at 37 °C, indicating that gel samples at physiological temperature supported controlled release of biologics. With the release of relatively hydrophilic tenofovir disoproxil, 65 % of drug was released after 8 h and drug release was independent of temperature [9]. The optimized formulation exhibited similar vaginal mucoadhesive profile with poloxamer 407 based delivery system and it was not toxic to human keratinocyte (HaCaT cells), based on MTS and LDH assay shown in Fig. 6 [9].

PNIPAM-b-PEG-b-PNIPAM systems have also been evaluated for vaginal delivery of progesterone and tenofovir [65]. Relative to poloxamer 407, these synthetic systems show much lower dependence of *Tgel* on concentration which is desirable for topical administration where fluid may be present. Dilution of poloxamer 407 gels has previously been shown to elevate *Tgel* and thus induce a gel-sol transition in vivo [66]. Furthermore PNIPAM-b-PEO-b-PNIPAM system has a greater resistance to dissolution than poloxamer 407 gels. Both these effects were attributed to the mechanisms of gelation of PNIPAM-b-PEO-b-PNIPAM, where PNIPAM LCST induces formation of spherical micelles which are attracted to each other by polymer bridges, reducing dissolution rates relative to poloxamer 407 which requires a highly concentrated face-centred cubic phase to form a gel [67].

### 3.5. Cervical formulations

Local drug delivery has been identified as an efficient strategy to treat superficial cervical cancer by instillation of drug formulations through the vagina to the cervix using a catheter [68]. Nevertheless, systemic therapies may be necessary for advanced forms of cervical cancer with metastatic and recurrent tendencies [69].

Xu et al formulated composite system of paclitaxel loaded MPEG-PCL polymeric micelles and cisplatin containing PEG-PCL-PEG based

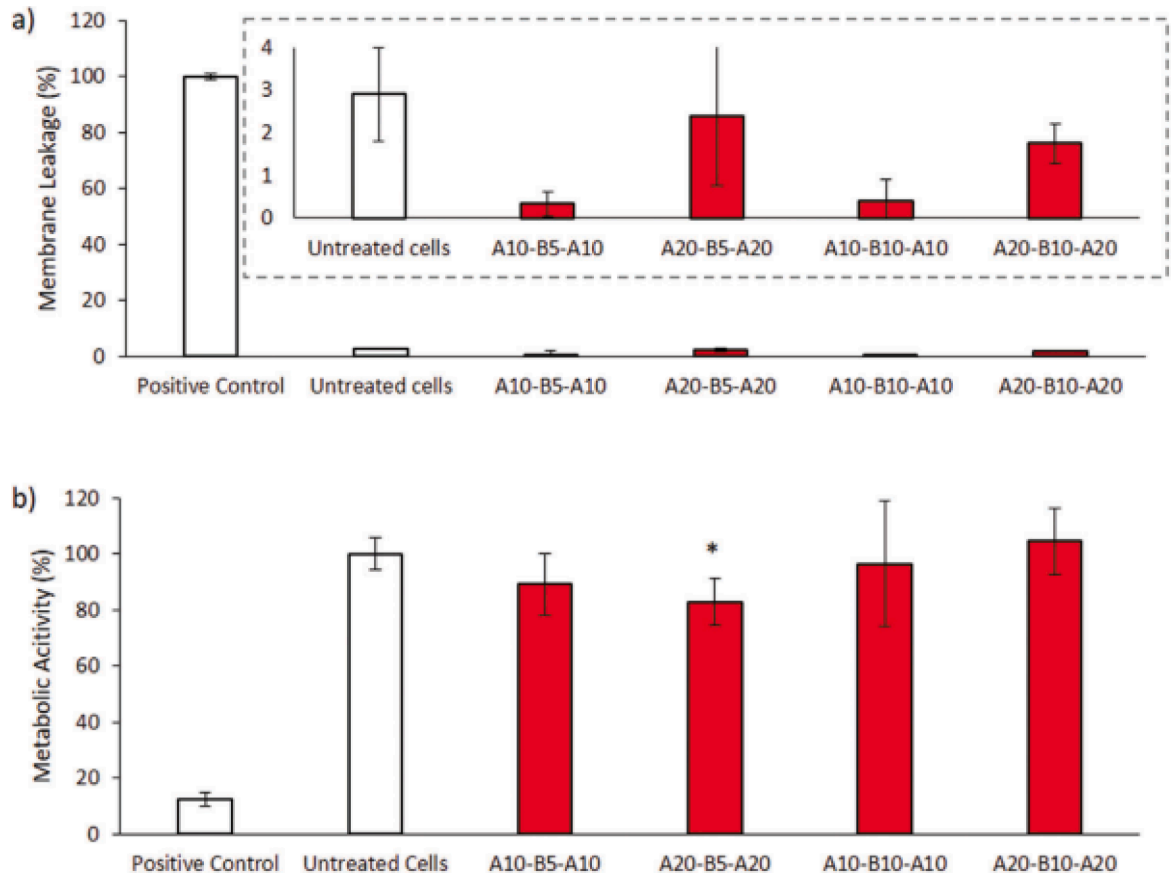


**Fig. 6.** Evaluation of polymer cytotoxicity by a) lactate dehydrogenase and b) formazan (MTS) assay showing membrane integrity and mitochondrial activity, respectively. Cells dosed with polymer solution (1% w/v) for 2 h prior to evaluation. Positive controls were treated with 0.1 % v/v Triton-X 100. Insert on (a) is expanded to show near-zero values. * designates statistical significance from untreated cells ($p < 0.05$) by one-way ANOVA with Tukey post-hoc (GraphPad Prism, USA). Data is presented as mean ± standard deviation ($n = 4$).
reproduced with permission from [9]

thermosensitive gel (PDMP), for potential treatment of locally advanced cervical cancer [57]. There was no remarkable difference in the gelation temperature of triblock copolymer-based hydrogels and composite system of hydrogels and polymeric micelles, indicating that incorporation of the nanoparticles into the hydrogels did not compromise their gelation potential at physiological temperature (GT ≈30 °C). In vivo studies revealed that the composite system of hydrogels and nanoparticles suppressed tumour development and the survival of tumour bearing mice was prolonged in comparison to cisplatin hydrogels or paclitaxel nanoparticles (55 days versus 40 days versus 50 days). PDMP was biocompatible based on histopathological analysis and H & E staining of sections of the mouse organs as there was no sign of organ toxicity [57].

In a latter study, a composite system of toad venom (TV) loaded solid lipid nanoparticles and nanorealgar containing poloxamer 188/P407(5 %/27.5 %)-based thermosensitive in situ gel were studied to improve sustained drug release profile; reduce systemic side effects and their vaginal irritation of toad venom and nanorealgar [58]. The gelation potential of composite system of nanoparticles and thermosensitive hydrogels was studied using tube inversion method at 37 °C and rheometer. The optimized formulation exhibited gelation temperature of 33 ± 0.9 °C. There was no remarkable difference in the gelation potential of the blank formulation after incorporation of the toad venom loaded solid lipid nanoparticles and nanorealgar powder. The drug formulation was stable at 4 °C for 3 months as well as being biocompatible based on histological studies of treated sections of rabbit vagina [58]. Fluorescent formulation was retained in the vagina of nude mice for 35 h [58]. The new formulation could improve patient compliance due to its controlled drug release profile; minimal vaginal irritation and reduced dosing frequency.

### 3.6. Intravesical formulations

Composite system of floatable doxorubicin loaded human serum albumin nanoparticles and poloxamer gel was investigated for potential bladder cancer treatment. Incorporation of nanoparticles into in situ hydrogels did not compromise its gelation potential as the doxorubicin nanoparticulate gel system (NP-Dox-Gel) exhibited a gelation temperature (GT) of 10 °C and gelation time (Gt) of 2 min at 37 °C while doxorubicin loaded nanoparticles and non-floating hydrogel displayed GT of 12 to 18 °C and Gt of 2–5 min at 37 °C [59]. NP-Dox-Gel was resistant to elimination during urine voiding and exhibited sustained drug release profile [59].

Amphiphilic N-[1-(2, 3-dioleoyloxy) propyl] - N, N, N-trimethylammonium chloride (DOTAP) is a mucoadhesive polymeric excipient that has been used to prepare FDA approved gene product [60]. Composite system of deguelin loaded DOTAP and monomethoxyl poly (ethylene glycol)-poly ε-caprolactone hybrid nanoparticles and Pluronic F127 based hydrogels (D/DMP-F gel) was studied for bladder cancer treatment. Fluorescent composite drug carrier was syringeable at 25 °C and gel was observed in the mice's bladder within 10 min of intravesical administration and residence of the D/DMP-F gel was sustained for 2 h [60]. Thus, the formulation may be safer, effective with sustained drug release profile due to its gradual elimination from the bladder.

Şenyiğit and co-workers prepared gemcitabine hydrochloride loaded chitosan-thioglycolic acid conjugate (CHI-TGA) nanoparticles (CHI-TGA NPs) and incorporated them into 2 % chitosan gel (CHI-TGA NPs/CHI)

or in situ gel forming poloxamer (CHI-TGA NPs/Plx gel) for improved intravesical treatment of bladder cancer [61]. The poloxamer based composite nanoparticulate hydrogel system was more syringeable than the chitosan-based formulations based on the work done to expel syringe content: CHI-TGA NPs/Plx gel (30.7 ± 1.4 Nmm) versus CHI-TGA NPs/CH gel (82.8 ± 0.8 Nmm). Using phosphate buffer medium (pH 6.5) as the release medium, the amount of drug released after 4 h study period from CHI-TGA NPs was greater than that of CHI-TGA NPs-CHI gel and CHI-TGA NPs-Plx gel (51.0 ± 3.7 % versus 33.4 % ± 5.0 % versus 19.6 % ±1.6 %). These findings indicated that poloxamer gel-based formulations exhibited superior sustained drug release profile relative to chitosan gel-based drug carriers. There was no significant statistical difference between the amount released within 4 h and 24 h for the studied formulations [61]. The studied formulations are presented in order of increasing urothelial mucosal drug permeation over 4 h: poloxamer gel based carrier (18.78 ± 1.97 %) < Gemcitabine solution (21.96 ± 1.20 %) < chitosan gel based carrier (33.16 ± 5.11 %) < CHI-TGA nanoparticles (37.32 ± 3.48 %), suggesting that thiolated chitosan based delivery systems may be preferred for improved drug delivery to underlying bladder cancerous tissues. The safety of all the studied formulations was confirmed based on the histopathological evaluation of treated bovine bladder mucosa, with no damage inflicted on healthy bladder tissues [61].

Kolawole and coworkers studied the syringeability, thermosensitivity, mucoadhesive and drug release properties of mitomycin-C containing chitosan/beta-glycerophosphate systems prepared using low (LCHIGP), medium (MCHIGP) and high molecular weight chitosan (HCHIGP), for potential intravesical treatment of bladder cancer [1]. Rheometry and vial inversion techniques were used to study the gelation potential of the formulations. The molecular weight of chitosan influenced the syringeable, in situ gelation, mucoadhesive and drug release profiles of the thermosensitive formulations. LCHIGP, MCHIGP and HCHIGP exhibited gelation temperature of 30.4 ± 0.3 °C, 29.8 ± 0.2 °C. and 29.6 ± 0.1 °C, respectively. Also, they displayed comparable gelation time based on rheology (1.6 min versus 1.4 min versus 1.0 min). However, HCHIGP displayed the fastest ease of gelation at 37 °C based on the vial inversion technique (gelation time of 5 ± 2 min). Also, HCHIGP displayed improved resistance to urine wash-out in comparison to MCHIGP and LCHIGP ($WO_{50}$ value of 6.1 ± 0.1 mL versus 7.9 ± 0.7 mL versus 9.3 ± 0.9 mL). The cumulative amount of mitomycin-C released after 6 h study period from LCHIGP, MCHIGP and HCHIGP was 63 ± 23%, 39 ± 20% and 37 ± 17%, respectively [1]. Overall, HCHIGP was the most promising formulation for intravesical treatment of bladder cancer.

Rapamycin loaded liposomes, composite system of rapamycin loaded unmodified liposomes/poloxamer gel (R-CL/P407) and rapamycin loaded folate modified liposomes/ poloxamer hydrogels (R-FL/P407) studied by Yoon et al exhibited similar gelation temperature and gelation time of 21 °C and 29 s, respectively [62]. Rapamycin was released from the liposomal gel formulations at a constant rate, with 100 % of rapamycin loaded liposomes released over 12 h whereas<5 % of rapamycin was released from the rapamycin liposome within similar time period, suggesting that drug loaded liposomal vesicles was released without molecular diffusion of the free drug [62]. R-FL/P407exhibited superior bladder tumour regression ability relative to unmodified rapamycin liposomes, folate modified liposomes and rapamycin solution (Fig. 7) [62]. These findings revealed that R-FL/ P407 facilitated targeted delivery of rapamycin to folate-receptor-expressing bladder cancer cells.

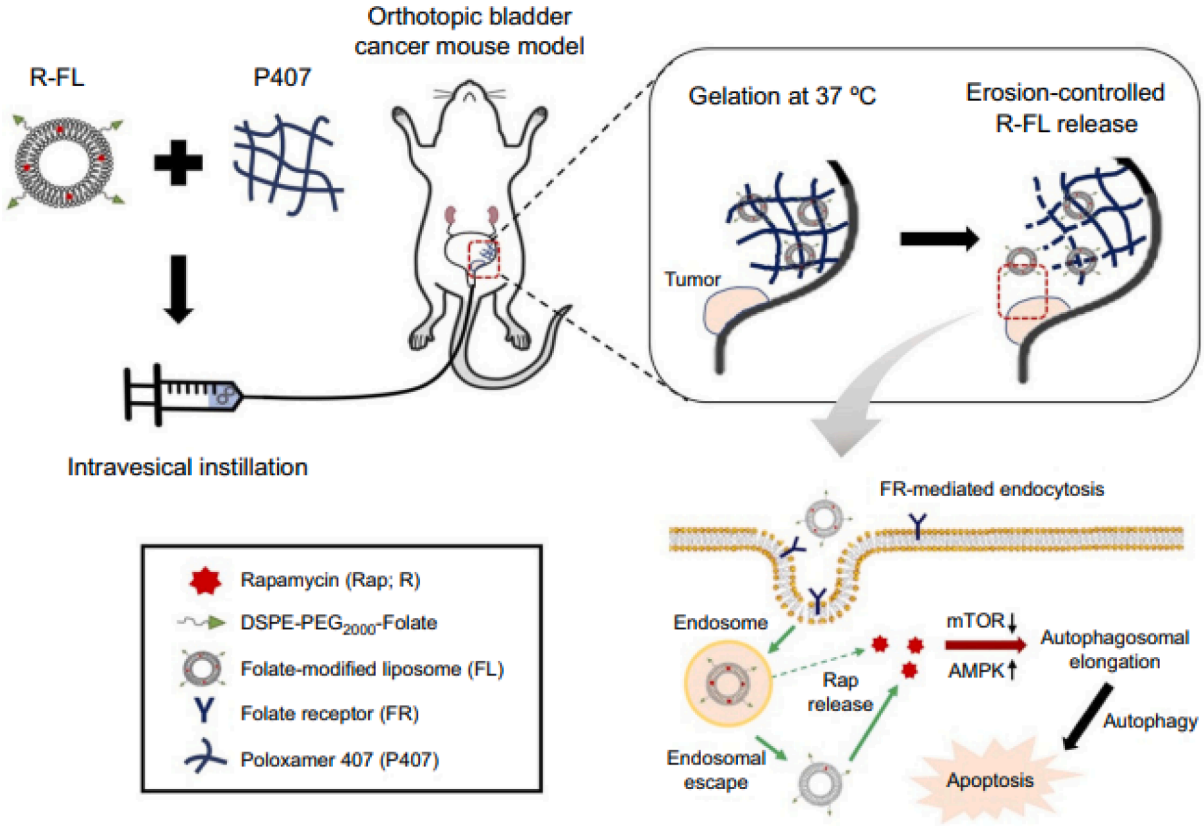


**Fig. 7.** Intravesical instillation of R-FL/P407 leading to gelation in the bladder, followed by erosion-controlled R-FL release, and enhanced absorption via FR-mediated endocytosis.Abbreviations: R-FL, rapamycin-loaded folate-modified liposome; P407, poloxamer 407; $DP_{2k}F$, distearoylphosphatidylethanolamine-polyethylene $glycol_{2000}$folate; FR, folate receptor [60].

## 4. Conclusions

In situ gelling drug delivery systems can prolong drug retention on mucosal surfaces in order to improve the therapeutic outcomes of patients. Drug or drug nanoparticles have been incorporated into stimuli-responsive gels to improve aqueous solubility, drug residence, controlled release profile, and bioavailability at specific body sites. The target mucosa dictates the type and concentration of in situ gelling and mucoadhesive polymers that would be used to formulate drug products.

It is desirable to formulate medicines intended for transmucosal application using highly mucoadhesive materials with sol–gel transition temperature between 30 °C and 37 °C in order to ensure that such drug delivery systems remain as liquid at room temperature to improve their syringeability and spreadability, though they would be mucoadhesive in nature and transform rapidly into gel at physiological temperature and adhere to mucosal surfaces for an extended period of time in order to reduce dosing frequency, improve therapeutic outcomes and acceptability of new in situ gelling formulations.

Critical in vitro studies required to evaluate the drug delivery applications of promising in situ gelling drug formulations include rheological studies; gelation temperature and gelation time evaluations; mucoadhesive evaluation in terms of the formulation resistance to biological fluid wash-out from freshly excised animal mucosa using fluorescence microscopy as well as measurement of force of detachment and work of adhesion using texture analyser. In vivo studies were also carried out on optimized formulations to confirm the most suitable combination of in situ gelling and mucoadhesive polymers for the drug of choice. In recent years, rheometry has been identified as an advanced, valuable and efficient technique required to predict the mechanical behavior of in situ gelling systems at various temperatures over time as well as their ability to withstand stress during processing.

A challenging limitation to the preclinical development of promising in situ gelling formulations from the research laboratory to the clinics is the lack of harmonized method for evaluating the syringeability, gelation potential, mucoadhesive and drug release profile of the studied drug formulations. Therefore, it is difficult to compare in vitro data across studies. Nevertheless, biocompatible and effective drug formulations have been identified based on clinical findings from in vitro and in vivo studies. Future efforts should push these systems towards clinical trial, however in the case of novel excipients, there are additional costs and risk associated with the concurrent toxicological evaluation of the excipient alone in addition to the medicine. Recent trials of streamlined regulatory pathways are being undertaken by the FDA which should catalyse the uptake of novel excipients into the portfolio of materials available to formulation scientists to unlock more efficacious medicines [70].

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

No data was used for the research described in the article.

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