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In SITU Gelling System as a Vaccine Carrier

Chandra HS[1], Basant Malik[3], Goutam Rath[2], Amit K. Goyal[2]

[1]Panacea Biotec Limited, Vaccine Formulation Plant, Baddi, Himachal Pradesh, India
[2]Nanomedicine Research Centre, Department of Pharmaceutics, ISF College of Pharmacy, Moga, Punjab, India
[3]Panacea Biotec Limited, Lalru, Punjab, India

ABSTRACT

The delivery of protein and peptides remain challenging task for the researchers in order to maintain integrity and stability of such molecules. Therefore, during formulation development phase it passes through various stress and loss their integrity due to configuration change. Thus, the quality of vaccine adjuvant plays a major role in addition to some other factors. Therefore, the adjuvant of formulation must satisfy compatibility issues apart from degree of immune stimulation. In this paper we are describing aluminum salt as adjuvant which is widely used in commercial available vaccine formulations. Despite immunomodulatory activity it has a lot of drawbacks viz. it is attractive but weak adjuvants, induces IgE production, allergenicity and neurotoxicity. In children it also causes azotemia and severe osteomalacia intoxication devoid of renal dialysis but it is only used in parenteral, and not for mucosal vaccination. In situ gelling system is most widely studied carrier system having characteristic of depot formation by different principle same as aluminum salt. The depot system is reported by various scientists for vaccine delivery and suggesting that it is good carrier for such types of sensitive material. In situ gelling system having various advantages viz. ease of administration and reduced frequency of administration, comfort and improved patient compliance. The material used for in situ gelling also provides lot of reward like biodegradable in nature, formulation stability, and particle size uniformity, sustained, prolonged and controlled release from depot, and biocompatibility characteristics. Those drugs which are sensitive to pH and enzymatic degradations it maintained potency of the drug and provides high interaction with tissues and biological fluids. Therefore, main advantage of it is that we can achieve mucosal as well as systemic immune response by administering antigen via. mucosal and non-mucosal sites. Thus, the use of in situ gel as vaccine adjuvant could be prevents infectious disease associated with pathogens.

Keywords: *In-situ* Gel, Depot System, Vaccine Adjuvant, Depot Forming Polymers

I. INTRODUCTION

To attain mass vaccination and complete eradications of infectious disease, there is utmost need to design and develop efficacious and safe vaccines. The quality of immune response depends upon various factors. Many strategies have been adopted for the improvement of the vaccine stability and efficacy. Among these, the use of vaccine adjuvant is most important factor, which influences quality of immune response. Aluminum salts are widely used adjuvant approved for human vaccines in the US, even though a diversity of novel adjuvants has been studied in the past few decades(Anderson 1997; Ulmer, DeWitt et al. 1999).

Aluminum hydroxide is widely used vaccine adjuvant which potentiates immune response by adsorbing antigen on its surface. It has quality to stimulate monocytes for the production of proinflammatory cytokines activating T cells. Due to the Activation of T-cell, Th2 cells release IL-4, which induces expression of MHC class II molecules on monocytes. The amplification in the expression of antigen-presenting and costimulatory molecules leads to enhanced accessory functions of monocytes(Ulanova,

Tarkowski et al. 2001). After vaccination, aluminum hydroxide act by depot formation at site of injection which allows slow release of antigen. Thus, it increases the interaction time between antigen immune cells i.e. macrophage, monocytes etc. Due to the formation of gel, aluminum hydroxide has ability to convert soluble antigens to particulate forms, which are then rapidly phagocytosed by immune cells(Gupta 1998).

Apart from this aluminum hydroxide has various drawbacks also, like it is only used in parenteral vaccine formulation not in mucosal vaccines. It is pretty weak adjuvant and rarely provokes cellular immune responses(Schirmbeck, Melber et al. 1994; Brewer, Conacher et al. 1996; Traquina, Morandi et al. 1996). It also induces IgE production, allergenicity(Audibert and Lise 1993; Goto, Kato et al. 1997) and neurotoxicity(Shaw, Li et al. 2013). At low doses aluminum are excreted by the kidneys under normal circumstances, which accumulate in the body and cause various toxicities. At high dose in the body, it predominately affects brain and bone tissues and thereby results in fatal neurological syndrome and dialysis-associated dementia and also cause amyotrophic lateral sclerosis and Alzheimer's disease(Petrovsky and Aguilar 2004). Large number of vaccines, alone and in combinations, having aluminum salts as adjuvant are being administered by children. In children when this adjuvant is given in a phosphate binder it enhances serum level of aluminum and causes azotemia and severe osteomalacia intoxication devoid of renal dialysis(Feldmann, Farber et al. 1992).

Large numbers of vaccine adjuvants have been studied from past few decades but they all were not successful for safe and effective delivery of antigen in humans largely because of toxicity, bioavailability, stability, and cost. And also because of physicochemical parameters like effects of size, electric charge and hydrophobicity which regulate the incorporation of proteins into the adjuvant formulation. Researchers also failed due to predictions on an empirical basis that which adjuvant will work most efficiently with a particular protein or peptide or specific carrier for particular antigen for better immune response(Petrovsky and Aguilar 2004).

In order to replace aluminum salt, in-situ gelling system might play as a helping hand overcoming such problems. It is successful in drug delivery and has lot of advantages like ease of administration and reduced frequency of administration, comfort and improved patient compliance(Liang, Baudouin et al. 2009). Diverse quality of polymers from both sources, natural and synthetic polymers are used which are biodegradable in nature. They provide sustained, prolonged and controlled release of the drug, good stability and biocompatibility characteristics, thereby making the *in situ* gel dosage forms more reliable. In situ gels have an attractive feature, which remains in sol form outside the body and changes to the gel form once administered in the body. Polymers used are having various advantages viz. maintain potency of the drug from pH and enzymes and provides high interaction with tissues and biological fluids. Use of polymers possesses formulation stability, particle size uniformity, control of drug release rate etc. From manufacturing point of view, large-scale manufacture of sterile preparations can be achieved, production method is simple, thus lowers the investment and manufacturing cost of final product(Sahoo, Sahoo et al. 2010) (Haynes, Tighe et al. 1999).

II. METHODS AND MATERIAL

Mechanism of Adjuvantcy

Aluminum salts act as antigen delivery systems by means of generating depots system that trap antigens at injection site, hence providing slow release of antigen in order to maintain stimulation of the immune response. Due to their particulate nature and optimal size (below 10μm) of carrier, they improve antigen perseverance at the site of injection sand increase staffing and activation of antigen presenting cells (APCs). By the activation of complement it stimulates immune competent cells of the body which further induces eosinophilia and activates macrophages (Gupta 1998). Aluminum salts have capability to bind antigens form multi-molecular aggregates which thereby evoke uptake by APCs. Iyer S et al. demonstrated that the Hepatitis B surface antigen (HBsAg) having lipid bilayer that is largely composed of phospholipids which differs it from many other antigens. Usually, phosphate groups adsorb powerfully to hydroxylated mineral surfaces through ligand exchange. Although, adsorption of HBsAg by aluminum hydroxide adjuvant is mainly due to ligand exchange among the phospholipids in HBsAg

and surface hydroxyls in aluminum hydroxide adjuvant(Iyer, Robinett et al. 2004).

Some adjuvants are also capable of directing antigen presentation by the major histocompatibility complexes (MHC)(Leroux-Roels 2010). Other adjuvants, basically ligands for pattern recognition receptors (PRR), act by means of induction of the innate immunity, predominantly targeting the APCs and therefore influencing the adaptative immune response. Members of nearly all of the PRR families are potential targets for adjuvants which include NOD-like receptors (NLRs), Toll-like receptors (TLRs), C-type lectin receptors (CLRs) and RIG-I-like receptors (RLRs). These receptors or signals act through distinct adaptor molecules important to the activation of different transcription factors. Once these factors (NF-κB, IRF3) are activated, they induce production of cytokines and chemokines which play a key role in the expansion, priming and polarization of the immune responses. When members of the NLR family (such as NLRP3 and NLRC4) are activated, they trigger the formation of a protein complex, called inflammasome, concerned in the generation of the pro-inflammatory cytokines IL-1β (Li, Willingham et al. 2008) and IL-18. The mechanism of action of inflammasomes NLRP3 and NLRC4, which have been involved in the innate immunity induced by certain adjuvantsstill remains unclear (Marrack, McKee et al. 2009) .

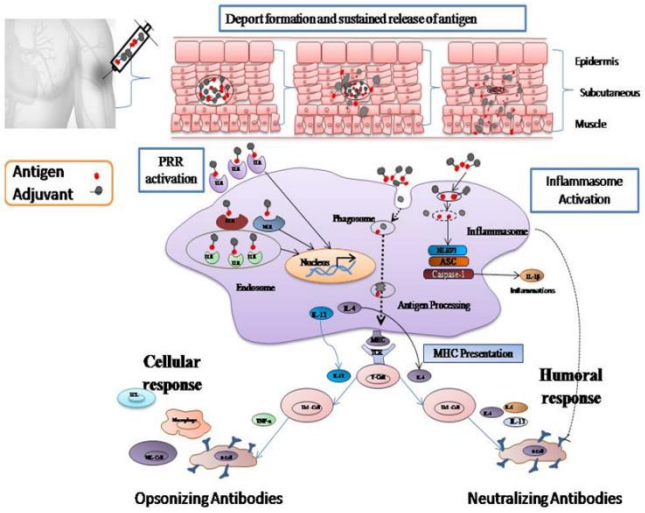

Figure 1 : Representations of mechanism of depot formations and activations of immune cells with immune response.

In situ gelling system has same gelling mechanism and adjutancy based on polymer characteristics. It has a lot of advantages in drug delivery systems. It also has great potential for antigen delivery via parenteral, as well as mucosal administrations. For example, pH sensitive liposomes have been successfully studied for in situ delivery system by Watarai et al. The polymer-modified liposomes have also been investigated as a carrier for vaccine delivery. For this mice were immunized via intraperitoneal route with ovalbumin-(OVA-) containing SucPG-modified liposomes. It was suggested that the pH-sensitive fusogenic polymer-(SucPG-) modified liposomes would serve effectively as an antigen delivery vehicle for inducing Th1 and Th2 immune responses.(Watarai, Iwase et al. 2013).

Tiwari et al. used liposomal in situ gelling system (LIGS) for mucosal vaccine delivery and concluded that it might overcome the major limitation of novel carriers with 100% entrapment of recombinant protein antigen, enhanced in vitro stability, good mucoadhesive property for prolonged retention in nasal cavity, prolonged antigen release and effective immunoadjuvant property(Tiwari, Goyal et al. 2009).

In Situ Gel as Antigen Delivery System

Researchers are finding and adopting various approaches how to prevent and eradicate infectious diseases thought the world. In this contrast lot of carriers have been study till date but we are not able to achieve maximum immune response. Experience with aluminum salt as adjuvant is that it helps to prevent infectious disease at some extent but not eradicate. Therefore, the replacement of such adjuvant is necessary, thus it can be replaced by in situ gelling system because shows same principle of depot formation. It is reported that this adjuvant provides maximum immune response with successful antigen delivery. Consequently, the pH sensitive liposomes have been successfully studied via in situ delivery system. Watarai et al studied polymer-modified liposomes as a carrier for vaccine delivery. For this mice were immunized through intraperitoneal route with ovalbumin- (OVA-) containing SucPG-modified liposomes. The inductions of OVA-specific antibody were observed in serum i.e. IgG1, IgG2a, and IgG3 Ab responses. IFN-γ-(Th1-type-) and IL-4-(Th2 type-) specific mRNA were also observed. It was suggested that the pH-sensitive fusogenic polymer-(SucPG-) modified liposomes would serve effectively as an antigen delivery vehicle for inducing Th1 and Th2 immune responses(Watarai, Iwase et al. 2013). Moreover, PLGA is FDA-approved polyester or co-

polymer which is widely studied for gene delivery but fails due to, too long release encapsulated payload and induction of high levels of target gene expression. Poly([beta]-amino ester) is a novel polymer, that is biodegradable and shows similar physical properties as PLGA. But poly([beta]-amino ester) shows pH-sensitive characteristic and acceptable for gene and vaccine delivery. The microparticles formulated by using these materials exhibited reasonably high DNA loadings and can considerably buffer the harsh acidic pH microenvironment produced by ester bond degradation. This material also showed immunomodulatory activity by activating antigen presenting cells in vitro and lead to antigen-specific, immune-mediated rejection of a lethal tumor dosage in vivo, a significant advance over conventional formulations(Little 2005). The encapsulation efficiency of proteins and peptides were improved by using Liposome in situ gelling system (LIGS). Humoral and mucosal immunity against Hepatitis B were evaluated using pH sensitive liposomes after nasal administration in Balb/c mice. The nasal mucosal response compared with alum-HBsAg vaccine injected intramuscularly. The results showed that alum-HBsAg vaccine did not elicit sIgA in mucosal secretions as it was induced and measured in the case of nasal administration of LIGS. Similarly, there was no cellular response (cytokine level) in case of alum-HBsAg vaccine. LIGS produced humoral (both systemic and mucosal) and cellular immune responses upon nasal administration.(Tiwari, Goyal et al. 2009)

Sustained release of thermosensitive chitosan hydrogel entrapped with liposomes and cubosomes were used to improve the efficacy of subunit vaccines and reduce the requirement for boosting. This system showed sustained release by forming depot at vaccine administration site were observed in vivo after antigen (OVA) loaded with chitosan thermogel and quil A as immunomodulator. The immunogenicity of chitosan thermogels containing cubosomes exhibited more stable lipidic particulate system and all gel-based formulations produced comparable effector immune responses in mice(Gordon, Young et al. 2012).Thermosensitive in situ gel system based on chitosan and poly vinyl alcohol (PVA) were developed by Agrawal et al. for nasal delivery of insulin. This system showed in situ gelation at 37^0C after 12 minutes of administrations. In conclusion, it was suggested that the successful vaccine delivery can be achieved by thermosensitive in situ gelling system(Agrawal, Gupta et al. 2010)

Enzymatic crosslinking is another approach used by Nicolynn E. et al. for in situ gelation) Recombinant human transglutaminase (hTG) and animal derived tissue transglutaminase (tTG enzymes were used for coupling two classes of protein polymers for enzymatic crosslinking along the protein. Utilizing tTG under physiological conditions, crosslinking occurred within two minutes, as determined by particle tracking microrheology. The properties of these gels were controlled through the specific nature of the protein polymer precursors, which renders these gels valuable for *in situ* therapies. Furthermore, the modular hydrogel composition allows tailoring of mechanical and physical properties for specific tissue engineering applications (Davis, Ding et al. 2010). Rapid gelling nasal inserts were developed for influenza vaccine using xanthan gum having negatively charged polymer, which additionally acts as immunomodulator by increasing serum IgG as well as the nasal IgA response in in-vivo. Poly-l-arginine and cationic lipid were found to be the best adjuvants. Inserts formulated from xanthan gum and cationic lipid stabilized with NaCl showed a reduced protein activity but were better than the cationic lipid alone. In vivo study on rat showed that xanthan gum containing the influenza vaccine, with or without an additional cationic lipid adjuvant, resulted in similar IgG levels as the pure nasal liquid vaccine formulation(Bertram, Bernard et al. 2010).

III. RESULTS AND DISCUSSION

APPROACHES FOR IN SITU GELLING POLYMERIC DRUG/PROTEIN DELIVERY SYSTEM

When polymers having diverse characteristics come in contact with physiological conditions, they form gels, and such approach called is known as in situ gelling. Physiological environmental conditions that induces different responses to form gels includes: light, electric Fields, magnetic fields, pressure, sound, physical stimuli such as change in temperature chemical stimuli such as change in pH and ion activation from biological fluids; and biological stimuli such as alteration in glucose level(Qiu and

Park 2001). The mechanism of depot formation is illustrated below;

A. Physiological Stimuli Approach

a. Temperature induced in situ gel system(Chen, Hou et al. 2006)

Various biomaterial have been studied whose transitions from sol-gel is triggered by raise in temperature and is an pretty perfect way to approach in situ gel formation. At ordinary temperature outside the body the in situ gelling system remains sol but when introduce in the body they turns in gel forms due to rise in temperature. Therefore, these hydrogels are liquid at room temperature (20 –25°C) and due to an increase in temperature they undergo gelation when comes in contact with body fluids (35 – 37°C), (Gong, Shi et al. 2009). For examples, Pluronics are poly (ethylene oxide)-poly(propylene oxide)-poly (ethylene oxide) (PEO-PPO-PEO) triblock co-polymers. It remains fluid at low temperature, but form thermoreversible gel when heated as a result of disorder-order transition in micelle packing which makes these polymers suitable for in situ gelation(Li, Zhu et al. 2008) others polymers like polyacrylamide (PAAm) or poly(acrylamide-co-butyl methacrylate) and poly(acrylic acid) (PAA) have positive temperature dependence of swelling(Owens, Jian et al. 2007).

b. pH induced in situ gel systems(Gupta, Singh et al. 2008)

Some polymers have tendency to form gel due to alteration in pH of the environment. Those polymers includes; poly(acrylic acid) (PAA) (Carbopol ®, carbomer), polymethacrilic acid (PMMA), cellulose acetate phthalate(CAP) latex and polyethylene glycol (PEG). These agents show pH-sensitive gelling property because they contain acidic or basic groups which either accept or release protons in response to changes in environmental pH. Sometimes the swelling of polymer causes rise in external pH in the case of weakly acidic (anionic) groups called polyacids. In addition to this, when it decrease polymer contains weakly basic (cationic) groups termed as polybases(Priya James, John et al. 2014).

B. Physical Change in Biomaterial Approach

Swelling mechanism: When material absorbs water from surrounding environment, it leads to swelling of material and turns into gel formations. Myverol 18-99 (glycerol mono-oleate) is polar lipid that swells in water to form lyotropic liquid crystalline phase structures(Shanbhag and Pandhare).

Diffusion mechanism: This principle act by precipitation or solidification of polymer matrix which involves diffusion of solvent from polymer solution into surrounding tissue. Solvents which follow this principle includes; dimethyl sulfoxide (DMSO), tertahydrofuran, 2-pyrrolidone N- methylpyrrolidone (NMP)(Kumar and Kapoor 2014).

C. Chemical reaction approach

Ionic Crosslinking

Carrageenan, sodium alginate, gellan gum (Gelrite) and pectin possess ionic charge on their surface. Due to the presence of ions these natural polysaccharides or polymers exhibits ionic sensitive and undergo phase transition with Mg2+, Ca2+, Na+ and K+.(Moura, Faneca et al. 2011)

Photo-polymerisation

Polymerization is the process of formation of polymer by addition of monomer units. When such approach is adopted inside the body, application of electromagnetic radiation is used to form gel after solution of monomers or reactive macromer and initiator can be injected into a tissue site. For example in the presence of suitable photo initiator the acrylate or similar polymerizable functional groups on the individual monomers and macromers quickly go through photopolymerisation. Light sensitive materials, when introduced to the desired site via injection it gets photocured in situ with optic fibre cables which extended drug release for longer time period. (Dulay, Choi et al. 2007; Winther-Jensen, Armel et al. 2010).

Enzymatic cross-linking

It is reported that in situ gel formation is catalysed by natural enzymes which have some advantages over

photochemical and chemical approaches. For instance, photo-polymerizations require various harmful substances monomers and initiators but enzymatic process operates efficiently under physiologic conditions. Intelligent stimuli-responsive delivery systems using hydrogels that can release insulin have been investigated(Shanbhag and Pandhare).

Polymers Used For In-Situ Gelling System

Various polymers have been studied for the formulation of *in situ* gels includes alginic acid, gellan gum, xyloglucan, chitosan, pectin, poly(DL-lactic acid), poly-caprolactone and poly(DL-lactide-co-glycolide) etc(Madan, Bajaj et al. 2009)**.** In situ gels possess numerous advantages viz. good stability, controlled, sustained and prolonged release of the drug, and biocompatibility characteristics which makes formulations reliable. The mechanism of depot formation and drug release is based on selection of the polymer. The use of water soluble and biodegradable polymers for the in situ gel formulations possesses more acceptable and excellent drug delivery systems. Large number of publications have been reported over the last few decades on temperature, pH, and ion sensitive in-situ gel(Kushwaha, Saxena et al. 2012).

Pluronics or Poloxamers (Pluronic ™ F 127)

Pluronics have been approved by the FDA for applications as food additives, and agricultural products, pharmaceutical ingredients as drug delivery carriers and as injectable systems for tissue engineering processes(Aguilar, Elvira et al. 2007). The pluronic triblock copolymers are accessible in a variety of grades differing in molecular mass and physical forms. They exhibits both types of properties hydrophilic as well as lipophilic, thus it also behaves as surfactant that makes them useful for industrial applications. Among other things, they can be used to increase the water solubility of hydrophobic, oily substances or otherwise increase the miscibility of two substances with different hydrophobic ties. Pluronic (PEO/PPO) at ratio of 2:1 are absorbed in the aqueous medium and form micellar structures over critical micellar concentration and it is also because of its thermosensitive nature. A 25-40% aqueous solution of this material will gel at about body temperature, and drug release from such a gel occurs over a period of up to one week. Poloxamer have been reported for protein and peptides (viz. urease, insulin, growth factors and bone morphogenic protein) delivery which shows stable and sustained release rate of drug over several hours (Jeong, Kim et al. 2002; Aguilar, Elvira et al. 2007).

The gel formed in combinations of Dextran/Pluronic Chao Lin et al. revealed that the pluronics concentration of 10% or 20 w/v% showed thermosensitive property with a temperature increase from 10 to 37 °C and he also concluded that thermosensitive injectable hydrogels show promise for biomedical applications(Lin, Zhao et al. 2010). Another study suggested that the sustained release of an anticancer drug (epirubicin) shows in vivo tumor growth inhibition. Oral dose provides better response compared with intravenous epirubicin solutions in CT-26 mouse colon adenocarcinoma bearing Balb/c mice(Lo, Hsu et al. 2013). In combinations of gelling polymers pluronics as in situ hydrogel enables the favourable resolution of cells and satisfactory cell delivery for muscle regeneration applications(Abdi, Choi et al. 2012). Ma WD et al. utilized pluronic-g-poly(acrylic acid) as copolymers for ocular drug delivery and confirmed in situ gels containing such copolymer might be helpful in prolonging the drug resident time and thus improve bioavailability of via delivery system(Ma, Xu et al. 2008).

Polyacrylic Acid (PAA)

It is a large-molecular-weight compound which is pH sensitive anionic polymer in nature. At neutral pH this polymer shows anionic character because, in aqueous solutions many of the side chains of PAA will lose their protons and acquire a negative charge. The polyacrylate is a dry product which formed microparticles of asymmetrical shape provides stability for extended periods. When these particles come in contact with water they quickly swell and absorb water, urine, or other aqueous solutions. PAA exhibits pH-sensitive in situ gel studied by LO YL et al. for sustained release of an anticancer drug and suggested it might be helpful for future cancer therapy (Lo, Hsu et al. 2013). Ketorolac tromethamine which is a non-steroidal anti-inflammatory drug (NSAID) were incorporated in polyacrylic acid (Carbopol® 934) as pH-triggered in situ gel for sustained ophthalmic delivery. Results suggested that it enhances bioavailability during its longer precorneal residence

time and capacity to produce sustained release of drug. (Nanjwade, Manjappa et al. 2009)

Cellulose Acetate Phthalate Latex

It is a useful polymer which provides sustained drug delivery due to its latex which is a free running solution at a Ph of 4.4 and undergoes coagulation when the pH is increase by the tear fluid to pH 7.4. At low pH of the preparation, it can elicit uneasiness in a number of patients(Le Bourlais, Treupel-Acar et al. 1995). The poly acrylic acid and its lightly cross-linked commercial forms (Polycarbophil and Carbopol) exhibits strong mucoadhesion Carbomer (Carbopol) a cross-linked acrylic acid polymer (PAA) (Bernkop-Schnürch and Krajicek 1998). It also shows pH induced phase transition as the pH is raised above its pKa of about 5.5(Rajas, Gounder et al. 2011). It is reported that different grades shows different character viz. carbopol 934 gel has the lowest cross-linking density, while Carbopol 940 have the highest and Carbopol 981 intermediate, higher the cross linking capacity more stiff is the gel produced. Combinations of polymers have also been used in order to reduce total polymer content and improve the gelling properties. For ocular drug delivery combination of Carbopol and a suitable viscosity enhancing polymers have been reported viz. HPMC or MC allows a reduction in the PAA concentration without comprising the in situ gelling properties.(Liu, Li et al. 2006; Singh, Bushetti et al. 2010; Wu, Liu et al. 2011)

Gallan Gum

Gallan gum has a tendency of gelation which is temperature dependent or actions induced. This gelation is concerned with a 3-D network by complexation with cation and hydrogen bonding with water. Oral topical *in situ* gels of clotrimazole based on the concept of pH triggered and ion activated systems have been reported. Gallan gum at concentration of 0.1-0.75% w/possesses in situ gelling property based on ion triggered system beside of hydroxylpropylmethylcelluose E50LV which was used to prolong the release rate of clotrimazole (0.1% w/v). The gellan gum transform into stiff gels when the pH was raised in the presence of monovalent/divalent cations. Due to this character of gallan gum , gel showed good sustained release from the formulation(Harish, Prabhu et al. 2009). The gellan gum successfully delivers various drugs like scopolamine hydrobromide (SCOP) for motion sickness through nasal route(Cao, Zhang et al. 2007) clindamycin for vaginal infections(Gupta and Sharma 2011) Famotidine via oral route(Chandra Mohan, Manjunatha et al. 2009)mometasone furoate via nasal administration(Cao, Ren et al. 2009).

Alginic Acid/ Sodium Alginate

It is a linear block copolymer polysaccharides consisting of β-D mannuronic acid and α-L-glucuronic acid residue by 1, 4-glycosidic linkages. Dilute aqueous solution of alginates formed gel after addition of di and trivalent metal ions by a supportive processes relating successive glucuronic filtrate in the α-L-glucuronic acid blocks of the alginate chain (Tobin, Cooper et al. 1984). Alginic acid is a good vehicle for ophthalmic formulation, because it exhibits favorable biological properties such as nontoxicity, biodegradability, mucoadhesive properties and ability to gel readily in the eye (Abraham, Furtado et al. 2009).

Yuejiang Liu, et al. developed gelrite, alginate, and gelrite/alginate solution act as ion-activated *in situ* gelling vehicle for ophthalmic delivery of matrine. He concluded that the mixture of 0.2% Gelrite and 0.6% alginate solutions can be used as an *in situ* gelling vehicle to enhance ocular retention. (Liu, Liu et al. 2010). Sodium alginate with CaCO3 formed in situ gel of Clarithromycin and Metronidazole Benzoate which suggested that sodium alginate situ gels showed oral sustained release of Clarithromycin and Metronidazole Benzoate.(Patel, Dadhani et al. 2011). Sodium alginate is also reported for ophthalmic delivery of anti-inflammatory drug diclofenac sodium, which formed gel based on pH alteration in the administration site. (Asasutjarit, Thanasanchokpibull et al. 2011).

Pectin

The pectin can be isolated from Aloe vera which is a biodegradable acidic carbohydrate polymer. Pectin is usually found in plant cell walls. It is a family of polysaccharides, in which the polymer backbone comprises α- (1-4)-D-galacturonic acid residue(Ni, Turner et al. 2004). Low methoxy pectins readily interacts with aqueous solution of free calcium ions to form gels which crosslink the galactironic acid chain.

While the gelation of pectin will occur in the presence of H+ ions, a source of divalent ions, normally calcium ion is necessary to produce the gel that are appropriate vehicles for drug delivery. Divalent cations in stomach, out of transition of pectin to gel state after it is administered orally(Matia-Merino, Lau et al. 2004). Calcium ion in the complexed form may be induced in the formulation for the initiation of pectin gelation. Sodium citrate might be added to the pectin solution form a multipart with most of the calcium ions being added in the formulation. Therefore, the formulation may remain in a fluid state (sol), awaiting the breakdown of the complex in the acidic environment of the stomach, where calcium ion causes pectin gelation(Besson, Yapo et al. 2014).

Kubo W et al. reported that the potential of pectin formulation with in situ gelling properties for the oral sustained delivery of paracetamol (acetaminophen). The formulations consisted of dilute aqueous solutions (1% to 2% w/v) of low methoxy pectin containing calcium ions in complexed form, which upon release in the acidic environment of the stomach caused gelation of the pectin(Kubo, Konno et al. 2004; Miyazaki, Murofushi et al. 2013). Theophylline and cimetidine oral sustained delivery achieved via in situ gelling pectin formulation which shown diffusion-controlled release of theophylline from 1, 1.5, and 2% w/v pectin gels(Kubo, Itoh et al. 2005).

Xanthan Gum

Xanthum gum is an anionic, high molecular weight, extra cellular polysaccharide formed by fermentation of gram negative bacterium called *Xanthomonas campestris*(Rojas, Nishidomi et al. 2013). It contains a trisaccharides side chain of β-D-mannose-β-D-glucoronicacid-α-D-mannose attached and cellulosic backbone (β-D-glucose residues) with alternating glucose residues of the main chain. Due to the presence of both pyruvic acid and glucoronic acid groups in the side chain it exhibits anionic character. It is useful in many ophthalmic compositions as a viscosity enhancing agent(Hoffman 2013). The xanthan gum shows thermosensitivity and can be simply gelled by heating to the gel-critical temperature and consequently cooling them under setting temperature. Xanthan gum, locust bean gum and a pharmaceutically active drug in said liquid medium express composition having a pH sensitive solution of a pharmaceutically acceptable medium. Various drugs that have been delivered using xanthan gum are, Linezolid, metronidazole benzoate and clarithromycin.(Patel, Dadhani et al. 2011).

Chitosan

Chitosan is a polycationic, biodegradable and thermosensitive polymer obtained through alkaline deacetylation of chitin, which is a natural component of crab shell and shrimp. It shows biocompatibility and pH dependent solubility which continue dissolved in aqueous solution up to a pH of 6.2. The soluble chitosan becomes precipitated when pH increases more than 6.2, which leads to the arrangement into hydrated gel forms. Certain polyol salts having single anionic charge groups (viz. glycerol, sorbitol and fructose) interacts with thermally sensitive pH dependent gel forming aqueous solutions, without any chemical modification or cross linking(Rinaudo 2006). Chitosan based thermosensitive pilocarpine in situ gels provides prolong pre-corneal-drug contact time for enhancing diffusion and improve ocular bioavailability. It was concluded that in situ gels of pilocarpine using chitosan showed thermosensitive characteristic and therefore, might be suitable alternate to conventional ocular drug delivery system(Venkatesh, Kamlesh L et al. 2013).

Chitosan in situ gel have also been studied in combination with carbopol based formulation loaded with Timolol Maleate which showed therapeutically efficacious and fickian (diffusion controlled) type of release behaviour over 24 h periods. It has also been reported that it is a viable alternative to conventional eye drops and can also prevent the rapid drainage as in case of liposomes(Gupta and Vyas 2010). Another study with timolol maleate were conducted using novel copolymer, poly(*N*-isopropylacrylamide)–chitosan (PNIPAAm–CS), which formed thermosensitive in situ gel and exhibits potential utilization for ocular drug delivery. Results suggest that it may improve the efficacy, bio-availability, and compliance of some eye drugs(Cao, Zhang et al. 2007). Ur-rehman T et al. suggested that when chitosan was used with poloxamer 407 and/or TPP it forms gel due to the micellization and gelation of polymer thus, it improved drug loading and retention(Ur-Rehman, Tavelin et al. 2011). Another combination of chitosan with hyaluronic acid was

studied by Tan H et al. for adipose tissue regeneration. The freeze-dried hydrogels incorporated insulin and enzymes resulted in the formation of a tighter network structure in composite hydrogels. The results of this study suggested that the biodegradable and glucose-responsive hydrogel may have potential uses in adipose tissue engineering applications(Tan, Rubin et al. 2010). This combination is also utilized for cartilage tissue engineering through injectable *in situ* gel. It was determined that the composite hydrogel support cell viability and retained chondrocytic morphology which provides injectable, composite hydrogels for tissue engineering applications(Tan, Rubin et al. 2010).

Xyloglucan

Xylocglucan is a polysaccharide derived from tamarind seeds and is composed of a (1-4)-β-glucan backbone chain, which has (1-2)-α-D xylose branches that are partially degraded by β- glactosidase. Therefore, the product exhibits thermosensitive reversible gelation by the lateral stacking of the rod like chains. Xyloglucan produces thermally reversible gel at body temperature due to galactose elimination which control degree of sol gel transition temperature(Cavalier, Lerouxel et al. 2008).

Xyloglucan have been used for oral sustained delivery of paracetamol from in situ gel reported by Miyazaki S et al. and it was suggested that polysaccharide xyloglucan exhibits gelation with dilute aqueous solutions studied in rabbit and rat stomachs when chilled solutions attained body temperature (Miyazaki, Endo et al. 2003). The ocular delivery of pilocarpine hydrochloride was achieved by in situ gel of xylocane. The miotic response of xyloglucan sol was studied in rabbit and compared with pluronic F127 sols containing the same drug concaentration. Similar results were obsorbed from miotic response after sustained release of pilocarpine from the 1.5% w/w xyloglucan gel and from 25% w/w Pluronic F127 gel(Miyazaki, Suzuki et al. 2001). Aqueous mixtures of xyloglucan 1.5% and 0.5% alginate had appropriate viscosities for ease of swallowing and suitable gelation temperatures to ensure in situ gelation following oral administration.(Itoh, Tsuruya et al. 2010) Xyloglucan proposed slow gelation time which provides potential application via oral route which allow in situ gelation in the stomach. Xyloglucan gels are widely studied for oral, intraperitoneal, ocular and rectal drug delivery(Carmen Chifiriuc, Mihai Grumezescu et al. 2014).

Poly Ethylene Glyocol (PEG)

Polyethylene glycol is biocompatible and hydrophilic polymer, and is available in different grades, which exhibit temperature dependent gelation in co-polymeric forms. For example, On hydration, poly(ethylene glycol)-poly(L-lactic acid)-poly(ethylene glycol) PEG-PLLA-PEG, triblock copolymers and PEG-PLLA is a diblock block copolymers demonstrate the sol-gel transition due to lowering in temperature. Degradation of the polymer matrix was slowed down by the incorporation of the PLGA blocks. (Jeong, Bae et al. 2000)

Mahoney and Anseth formed PEG hydrogels by photopolymerizing methacrylate groups covalently linked to degradable PEG macromers. Hydrogel degradation was monitored over time by measuring mechanical strength (compressive modulus) and average mesh size from swelling ratio data(Mahoney and Anseth 2006). Initially, the polymer chains were highly cross-linked, but as degradation proceeded, ester bonds were hydrolyzed, allowing the gel to swell; the compressive modulus decreased as the mesh size increased until the hydrogel was completely dissolved. It was demonstrated that neural precursor cells were able to be photoencapsulated and cultured on the PEG gels with minimal cell death. Because the mesh size is initially small, the hydrogel blocks inflammatory and other inhibitory signals from surrounding tissue. As the mesh size increases, the hydrogel is able to serve as a scaffold for axon regeneration(Haile, Haastert et al. 2007).

Masson C et al. demonstrated pH-sensitive PEG lipids which might be potential tools for nonviral gene delivery. It was found that pH-sensitive PEG lipids stabilized cationic lipid/DNA isoelectric complexes as efficiently as their non-pH-sensitive PEG analogs at neutral pH. Lowering the pH resulted in the precipitation of the complexes bearing pH-sensitive PEG lipids as a consequence of their degradation.(Masson, Garinot et al. 2004). Sargeant TD et al. developed a unique injectable hydrogel system composed of collagen and multi-armed poly(ethylene glycol) (PEG) . The collagen component

enables cellular adhesion and permits enzymatic degradation, while the multi-armed PEG component has amine-reactive chemistry that also binds proteins/tissue and is hydrolytically degradable. Data suggested that these collagen and PEG hydrogels exhibited the mechanical, physical and biological properties, which would be suitable for use as an injectable tissue scaffold for the treatment of a variety of simple and complex tissue defects(Sargeant, Desai et al. 2012).

Khodaverdi E et al. used naltrexone hydrochloride and vitamin B_{12} as drug incorporated in copolymers PCL-PEG-PCL hydrogels(Khodaverdi, Golmohammadian et al. 2012). Poly(ε-caprolactone)–poly(ethylene glycol)–poly(ε-caprolactone) (PCEC) used as in situ gel due to its thermosensitivity for controlled drug delivery after injection(Wang, Deng et al. 2012). It was suggested that the model drugs could be released from the PCEC hydrogel system for longer time periods. PCEC hydrogel is promising for use as an injectable local drug delivery system(Gong, Shi et al. 2009).

Myverol 18- 99 (glycerol monooleate)

Myverol 18- 99 (glycerol monooleate) is polar lipid that swells in water to form lyotropic liquid crystalline phase structures(Mengesha, Wydra et al. 2013). In situ formation based on Physical mechanism Swelling(Patel, Chauhan et al. 2012). Therefore, myverol 18- 99 act via diffusion of solvent from into surrounding tissue and results in precipitation or solidification of polymer matrix. N- methyl pyrrolidone (NMP) have been shown to be good solvent for gelling by such system.

IV. CONCLUSION

Firstly, the vaccine adjuvant must be satisfied regulatory requirement, maintained vaccine efficacy and antigen stability. Aluminum salt approved by US/FDA and squalene as emulsion adjuvant for animals and emergency use for humans are only adjuvants for vaccine delivery. It is important that the selection of appropriate adjuvant for specific antigen because every adjuvant has different degree of immune stimulation. Mostly the immune response influences by antigen combinations or by adjuvant combinations and some other factors. As mention above in situ gelling system has various advantages thus it may be further study for antigen delivery and might be successful. Therefore, In situ gelling system might be use for successful vaccine delivery and get maximum immune response via mucosal and parenteral vaccinations.

V. REFERENCES

[1] Abdi, S. I. H., J. Y. Choi, et al. (2012). "In Vivo study of a blended hydrogel composed of pluronic F-127-alginate-hyaluronic acid for its cell injection application." Tissue Engineering and Regenerative Medicine 9(1): 1-9.

[2] Abraham, S., S. Furtado, et al. (2009). "Sustained ophthalmic delivery of ofloxacin from an ion-activated in situ gelling system." Pakistan journal of pharmaceutical sciences 22(2).

[3] Agrawal, A., P. Gupta, et al. (2010). "Development and characterization of in situ gel system for nasal insulin delivery." Die Pharmazie-An International Journal of Pharmaceutical Sciences 65(3): 188-193.

[4] Aguilar, M., C. Elvira, et al. (2007). "Smart polymers and their applications as biomaterials." Topics in tissue engineering 3: 1-27.

[5] Anderson, D. P. (1997). "Adjuvants and immunostimulants for enhancing vaccine potency in fish." Dev Biol Stand 90: 257-265.

[6] Asasutjarit, R., S. Thanasanchokpibull, et al. (2011). "Optimization and evaluation of thermoresponsive diclofenac sodium ophthalmic in situ gels." Int J Pharm 411(1-2): 128-135.

[7] Audibert, F. M. and L. D. Lise (1993). "Adjuvants: current status, clinical perspectives and future prospects." Trends Pharmacol Sci 14(5): 174-178.

[8] Bernkop-Schnürch, A. and M. E. Krajicek (1998). "Mucoadhesive polymers as platforms for peroral peptide delivery and absorption: synthesis and evaluation of different chitosan–EDTA conjugates." Journal of controlled release 50(1): 215-223.

[9] Bertram, U., M. C. Bernard, et al. (2010). "In situ gelling nasal inserts for influenza vaccine delivery." Drug Dev Ind Pharm 36(5): 581-593.

[10] Besson, V., B. M. Yapo, et al. (2014). "Macromolecular and Viscoelastic Properties of

Low Methoxy Pectin from Cashew Apple Pomace."

[11] Bilensoy, E., M. A. Rouf, et al. (2006). "Mucoadhesive, thermosensitive, prolonged-release vaginal gel for clotrimazole: β-cyclodextrin complex." AAPS PharmSciTech 7(2): E54-E60.

[12] Brewer, J. M., M. Conacher, et al. (1996). "In interleukin-4-deficient mice, alum not only generates T helper 1 responses equivalent to freund's complete adjuvant, but continues to induce T helper 2 cytokine production." Eur J Immunol 26(9): 2062-2066.

[13] Cao, S.-l., X.-w. Ren, et al. (2009). "In situ gel based on gellan gum as new carrier for nasal administration of mometasone furoate." International journal of pharmaceutics 365(1): 109-115.

[14] Cao, S.-l., Q.-z. Zhang, et al. (2007). "Preparation of ion-activated in situ gel systems of scopolamine hydrobromide and evaluation of its antimotion sickness efficacy." Acta Pharmacologica Sinica 28(4).

[15] Cao, Y., C. Zhang, et al. (2007). "Poly (< i> N</i>-isopropylacrylamide)–chitosan as thermosensitive in situ gel-forming system for ocular drug delivery." Journal of controlled release 120(3): 186-194.

[16] Carmen Chifiriuc, M., A. Mihai Grumezescu, et al. (2014). "Biomedical Applications of Natural Polymers for Drug Delivery." Current Organic Chemistry 18(2): 152-164.

[17] Cavalier, D. M., O. Lerouxel, et al. (2008). "Disrupting two Arabidopsis thaliana xylosyltransferase genes results in plants deficient in xyloglucan, a major primary cell wall component." The Plant Cell Online 20(6): 1519-1537.

[18] Chen, G., S. X. Hou, et al. (2006). "[In vivo distribution and pharmacokinetics of dexamethasone sodium phosphate thermosensitive in situ gel following intratympanic injection]." Sichuan Da Xue Xue Bao Yi Xue Ban 37(3): 456-459.

[19] Davis, N. E., S. Ding, et al. (2010). "Modular enzymatically crosslinked protein polymer hydrogels for in situ gelation." Biomaterials 31(28): 7288-7297.

[20] Dulay, M. T., H. N. Choi, et al. (2007). "Visible light‐induced photopolymerization of an in situ macroporous sol‐gel monolith." Journal of separation science 30(17): 2979-2985.

[21] Feldmann, B., D. Farber, et al. (1992). "[Aluminum poisoning caused by the phosphate binder in a non-dialysed child with chronic renal insufficiency]." Radiologe 32(7): 327-332.

[22] Gong, C., S. Shi, et al. (2009). "Biodegradable in situ gel-forming controlled drug delivery system based on thermosensitive PCL–PEG–PCL hydrogel. Part 2: Sol–gel–sol transition and drug delivery behavior." Acta biomaterialia 5(9): 3358-3370.

[23] Gordon, S., K. Young, et al. (2012). "Chitosan hydrogels containing liposomes and cubosomes as particulate sustained release vaccine delivery systems." Journal of liposome research 22(3): 193-204.

[24] Goto, N., H. Kato, et al. (1997). "Local tissue irritating effects and adjuvant activities of calcium phosphate and aluminium hydroxide with different physical properties." Vaccine 15(12-13): 1364-1371.

[25] Gupta, H. and A. Sharma (2011). "Ion activated bioadhesive in situ gel of clindamycin for vaginal application." International journal of drug Delivery 1(1).

[26] Gupta, H., R. M. Singh, et al. (2008). "pH-Induced In Situ Gel for Periodontal Anesthesia." Indian J Pharm Sci 70(6): 776-778.

[27] Gupta, R. K. (1998). "Aluminum compounds as vaccine adjuvants." Advanced Drug Delivery Reviews 32(3): 155-172.

[28] Gupta, S. and S. P. Vyas (2010). "Carbopol/chitosan based pH triggered in situ gelling system for ocular delivery of timolol maleate." Sci Pharm 78(4): 959-976.

[29] Haile, Y., K. Haastert, et al. (2007). "Culturing of glial and neuronal cells on polysialic acid." Biomaterials 28(6): 1163-1173.

[30] Harish, N. M., P. Prabhu, et al. (2009). "Formulation and Evaluation of in situ Gels Containing Clotrimazole for Oral Candidiasis." Indian J Pharm Sci 71(4): 421-427.

[31] Haynes, R. J., P. J. Tighe, et al. (1999). "Antimicrobial defensin peptides of the human ocular surface." Br J Ophthalmol 83(6): 737-741.

[32] Hoffman, A. S. (2013). "Stimuli-responsive polymers: Biomedical applications and

challenges for clinical translation." Advanced Drug Delivery Reviews 65(1): 10-16.
[33] Itoh, K., R. Tsuruya, et al. (2010). "In situ gelling xyloglucan/alginate liquid formulation for oral sustained drug delivery to dysphagic patients." Drug Dev Ind Pharm 36(4): 449-455.
[34] Iyer, S., R. Robinett, et al. (2004). "Mechanism of adsorption of hepatitis B surface antigen by aluminum hydroxide adjuvant." Vaccine 22(11): 1475-1479.
[35] Jeong, B., Y. H. Bae, et al. (2000). "Drug release from biodegradable injectable thermosensitive hydrogel of PEG–PLGA–PEG triblock copolymers." Journal of controlled release 63(1): 155-163.
[36] Jeong, B., S. W. Kim, et al. (2002). "Thermosensitive sol–gel reversible hydrogels." Advanced Drug Delivery Reviews 54(1): 37-51.
[37] Khodaverdi, E., A. Golmohammadian, et al. (2012). "Biodegradable in situ gel-forming controlled drug delivery system based on thermosensitive poly (-caprolactone)-poly (ethylene glycol)-poly (-caprolactone) hydrogel." ISRN pharmaceutics 2012.
[38] Kubo, W., K. Itoh, et al. (2005). "Oral sustained delivery of theophylline and cimetidine from in situ gelling pectin formulations in rabbits." Drug Dev Ind Pharm 31(8): 819-825.
[39] Kubo, W., Y. Konno, et al. (2004). "In situ gelling pectin formulations for oral sustained delivery of paracetamol." Drug Dev Ind Pharm 30(6): 593-599.
[40] Kumar, D. and P. Kapoor (2014). "An Insight to In-Situ Gel Forming Stomach Specific Drug Delivery System." PharmaTutor 2(2): 25-32.
[41] Kushwaha, S. K., P. Saxena, et al. (2012). "Stimuli sensitive hydrogels for ophthalmic drug delivery: A review." Int J Pharm Investig 2(2): 54-60.
[42] Le Bourlais, C., L. Treupel-Acar, et al. (1995). "New ophthalmic drug delivery systems." Drug development and industrial pharmacy 21(1): 19-59.
[43] Leroux-Roels, G. (2010). "Unmet needs in modern vaccinology: adjuvants to improve the immune response." Vaccine 28 Suppl 3: C25-36.
[44] Li, H., S. B. Willingham, et al. (2008). "Cutting edge: inflammasome activation by alum and alum's adjuvant effect are mediated by NLRP3." J Immunol 181(1): 17-21.
[45] Li, X. Y., Z. J. Zhu, et al. (2008). "[Characteristics of poloxamer thermosensitive in situ gel of dexamethasone sodium phosphate]." Yao Xue Xue Bao 43(2): 208-213.
[46] Liang, H., C. Baudouin, et al. (2009). "Comparison of the ocular tolerability of a latanoprost cationic emulsion versus conventional formulations of prostaglandins: an in vivo toxicity assay." Mol Vis 15: 1690-1699.
[47] Lin, C., P. Zhao, et al. (2010). "Thermosensitive in situ-forming dextran–pluronic hydrogels through Michael addition." Materials Science and Engineering: C 30(8): 1236-1244.
[48] Little, S. S. R. (2005). Poly ([beta]-amino ester) s as pH sensitive biomaterials for microparticulate genetic vaccine delivery, Massachusetts Institute of Technology.
[49] Liu, Z., J. Li, et al. (2006). "Study of an alginate/HPMC-based in situ gelling ophthalmic delivery system for gatifloxacin." International journal of pharmaceutics 315(1): 12-17.
[50] Lo, Y. L., C. Y. Hsu, et al. (2013). "pH-and thermo-sensitive pluronic/poly(acrylic acid) in situ hydrogels for sustained release of an anticancer drug." J Drug Target 21(1): 54-66.
[51] Ma, W. D., H. Xu, et al. (2008). "Temperature-responsive, Pluronic-g-poly(acrylic acid) copolymers in situ gels for ophthalmic drug delivery: rheology, in vitro drug release, and in vivo resident property." Drug Dev Ind Pharm 34(3): 258-266.
[52] Madan, M., A. Bajaj, et al. (2009). "In situ forming polymeric drug delivery systems." Indian J Pharm Sci 71(3): 242-251.
[53] Mahoney, M. J. and K. S. Anseth (2006). "Three-dimensional growth and function of neural tissue in degradable polyethylene glycol hydrogels." Biomaterials 27(10): 2265-2274.
[54] Marrack, P., A. S. McKee, et al. (2009). "Towards an understanding of the adjuvant action of aluminium." Nat Rev Immunol 9(4): 287-293.
[55] Masson, C., M. Garinot, et al. (2004). "pH-sensitive PEG lipids containing orthoester linkers: new potential tools for nonviral gene delivery." Journal of controlled release 99(3): 423-434.
[56] Matia-Merino, L., K. Lau, et al. (2004). "Effects of low-methoxyl amidated pectin and ionic calcium on rheology and microstructure of acid-

induced sodium caseinate gels." Food Hydrocolloids 18(2): 271-281.
[57] Mengesha, A. E., R. J. Wydra, et al. (2013). "Binary Blend of Glyceryl Monooleate and Glyceryl Monostearate for Magnetically Induced Thermo-Responsive Local Drug Delivery System." Pharmaceutical research 30(12): 3214-3224.
[58] Miyazaki, S., K. Endo, et al. (2003). "Oral sustained delivery of paracetamol from in situ gelling xyloglucan formulations." Drug Dev Ind Pharm 29(2): 113-119.
[59] Miyazaki, S., H. Murofushi, et al. (2013). "The influence of the degree of esterification on the release characteristics of in situ gelling pectin formulations for oral sustained delivery of paracetamol." Pharm Dev Technol 18(5): 1259-1264.
[60] Miyazaki, S., S. Suzuki, et al. (2001). "In situ gelling xyloglucan formulations for sustained release ocular delivery of pilocarpine hydrochloride." Int J Pharm 229(1-2): 29-36.
[61] Mottu, F., P. Gailloud, et al. (2000). "In vitro assessment of new embolic liquids prepared from preformed polymers and water-miscible solvents for aneurysm treatment." Biomaterials 21(8): 803-811.
[62] Moura, M. J., H. Faneca, et al. (2011). "In situ forming chitosan hydrogels prepared via ionic/covalent co-cross-linking." Biomacromolecules 12(9): 3275-3284.
[63] Nanjwade, B. K., A. Manjappa, et al. (2009). "A novel pH-triggered in situ gel for sustained ophthalmic delivery of ketorolac tromethamine." Asian J Pharm Sci 4(3): 189-199.
[64] Ni, Y., D. Turner, et al. (2004). "Isolation and characterization of structural components of< i> Aloe vera</i> L. leaf pulp." International Immunopharmacology 4(14): 1745-1755.
[65] Owens, D. E., Y. Jian, et al. (2007). "Thermally responsive swelling properties of polyacrylamide/poly (acrylic acid) interpenetrating polymer network nanoparticles." Macromolecules 40(20): 7306-7310.
[66] Patel, R., B. Dadhani, et al. (2011). "Formulation, evaluation and optimization of stomach specific in situ gel of clarithromycin and metronidazole benzoate." International journal of drug Delivery 2(2).
[67] Patel, R. B., M. A. Chauhan, et al. (2012). "Floating In Situ Gel: New Trends in Controlled and Sustained Gastroretentive Drug Delivery System." Research Journal of Pharmacy and Technology 5(7): 889-893.
[68] Petrovsky, N. and J. C. Aguilar (2004). "Vaccine adjuvants: current state and future trends." Immunol Cell Biol 82(5): 488-496.
[69] Priya James, H., R. John, et al. (2014). "Smart polymers for the controlled delivery of drugs–a concise overview." Acta Pharmaceutica Sinica B.
[70] Qiu, Y. and K. Park (2001). "Environment-sensitive hydrogels for drug delivery." Advanced Drug Delivery Reviews 53(3): 321-339.
[71] Rajas, N. J., T. Gounder, et al. (2011). "In situ opthalmic gels: a developing trend." International Journal of Pharmaceutical Sciences Review & Research 7(1).
[72] Rinaudo, M. (2006). "Chitin and chitosan: properties and applications." Progress in polymer science 31(7): 603-632.
[73] Rojas, R., S. Nishidomi, et al. (2013). "Glutamate transport and xanthan gum production in the plant pathogen Xanthomonas axonopodis pv. citri." World Journal of Microbiology and Biotechnology 29(11): 2173-2180.
[74] Rozier, A., C. Mazuel, et al. (1989). "Gelrite< sup>®</sup>: A novel, ion-activated, in-situ gelling polymer for ophthalmic vehicles. Effect on bioavailability of timolol." International journal of pharmaceutics 57(2): 163-168.
[75] Sahoo, S., R. Sahoo, et al. (2010). "Mucoadhesive nanopolymer-A novel drug carrier for topical ocular drug delivery." Eur J Sci Res 46: 401-409.
[76] Sargeant, T. D., A. P. Desai, et al. (2012). "An in situ forming collagen-PEG hydrogel for tissue regeneration." Acta Biomater 8(1): 124-132.
[77] Schirmbeck, R., K. Melber, et al. (1994). "Antibody and cytotoxic T-cell responses to soluble hepatitis B virus (HBV) S antigen in mice: implication for the pathogenesis of HBV-induced hepatitis." J Virol 68(3): 1418-1425.
[78] Shanbhag, P. P. and P. P. Pandhare "In situ gelling polymeric drug delivery system."
[79] Shaw, C. A., Y. Li, et al. (2013). "Administration of aluminium to neonatal mice

in vaccine-relevant amounts is associated with adverse long term neurological outcomes." J Inorg Biochem 128: 237-244.

[80] Singh, V., S. S. Bushetti, et al. (2010). "Stimuli-sensitive hydrogels: a novel ophthalmic drug delivery system." Indian J Ophthalmol 58(6): 477-481.

[81] Tan, H., J. P. Rubin, et al. (2010). "Injectable in situ forming biodegradable chitosan-hyaluronic acid based hydrogels for adipose tissue regeneration." Organogenesis 6(3): 173-180.

[82] Tiwari, S., A. K. Goyal, et al. (2009). "Liposome in situ gelling system: Novel carrier based vaccine adjuvant for intranasal delivery of recombinant protein vaccine." Procedia in Vaccinology 1(1): 148-163.

[83] Tobin, J. M., D. Cooper, et al. (1984). "Uptake of metal ions by Rhizopus arrhizus biomass." Applied and Environmental Microbiology 47(4): 821-824.

[84] Traquina, P., M. Morandi, et al. (1996). "MF59 adjuvant enhances the antibody response to recombinant hepatitis B surface antigen vaccine in primates." J Infect Dis 174(6): 1168-1175.

[85] Ulanova, M., A. Tarkowski, et al. (2001). "The Common vaccine adjuvant aluminum hydroxide up-regulates accessory properties of human monocytes via an interleukin-4-dependent mechanism." Infect Immun 69(2): 1151-1159.

[86] Ulmer, J. B., C. M. DeWitt, et al. (1999). "Enhancement of DNA vaccine potency using conventional aluminum adjuvants." Vaccine 18(1-2): 18-28.

[87] Ur-Rehman, T., S. Tavelin, et al. (2011). "Chitosan in situ gelation for improved drug loading and retention in poloxamer 407 gels." Int J Pharm 409(1-2): 19-29.

[88] VENKATESH, M., P. KAMLESH L, et al. (2013). "DEVELOPMENT AND EVALUATION OF CHITOSAN BASED THERMOSENSITIVE IN SITU GELS OF PILOCARPINE." International Journal of Pharmacy & Pharmaceutical Sciences 5(1).

[89] Wang, W., L. Deng, et al. (2012). "Adjustable degradation and drug release of a thermosensitive hydrogel based on a pendant cyclic ether modified poly (ε-caprolactone) and poly (ethylene glycol) co-polymer." Acta biomaterialia 8(11): 3963-3973.

[90] Watarai, S., T. Iwase, et al. (2013). "Efficiency of pH-Sensitive Fusogenic Polymer-Modified Liposomes as a Vaccine Carrier." The Scientific World Journal 2013.s

[91] Winther‐Jensen, O., V. Armel, et al. (2010). "In situ photopolymerization of a gel ionic liquid electrolyte in the presence of iodine and its use in dye sensitized solar cells." Macromolecular rapid communications 31(5): 479-483.

[92] Wu, H., Z. Liu, et al. (2011). "Design and evaluation of baicalin-containing in situ pH-triggered gelling system for sustained ophthalmic drug delivery." International journal of pharmaceutics 410(1): 31-40.