Sigma J Eng & Nat Sci 34 (3), 2016, 439-451

Sigma Journal of Engineering and Natural Sciences
Sigma Mühendislik ve Fen Bilimleri Dergisi

Review Paper / Derleme Makalesi

POLYMERS IN VACCINE FORMULATION

Zeynep MUSTAFAEVA*

Yıldız Teknik Üniversitesi, Kimya Metalurji Fakültesi, Biyomühendislik Bölümü, Esenler-İSTANBUL

Received/Geliş: 05.04.2016 Revised/Düzeltme: 16.05.2016 Accepted/Kabul: 14.07.2016

ABSTRACT

Synthetic peptides that bind to carrier polymer molecules to use recent vaccine technology can create a higher immune response, remain longer in biological systems and they may be more stable against degrading. This is why synthetic peptides are given to living systems either by creating a microatmosphere or a conjugate. In these systems where natural or synthetic polymers can be used; synthetic carriers have more advantage compared to natural carriers such as obtainingproducts with high purity yields, manipulating structure and the molecular weight as desired, creating specific effects with simple additions of various chemical groups to the structure.
In this study, linear and spherical polymeric carrier were examined for synthetic peptide, several examples are given to polymeric carrier and examined immune response and their advantage.
Keywords: Polymeric carriers, polyelectrolytes, nanoparticles.

AŞI FORMÜLASYONUNDA POLİMERLER

ÖZ

Polimerik taşıyıcı sistemler son dönemde aşı teknolojisinde sıklıkla kullanılan sentetik peptidlere, daha yüksek immün cevap oluşturabilme, biyolojik sistemde daha uzun süre kalabilme ve bozucu etkenlere karşı daha kararlı olabilme gibi avantajlar sağlar. Bu yüzden sentetik peptidler canlı sistemlere taşıyıcı mikroatmosferinde veya konjugat oluşturularak verilir. Doğal veya sentetik polimerlerin kullanıldığı bu sistemlerde sentetik polimerler yüksek saflıkta ürün eldesi, istenilen yapıda ve molekül ağırlığında sentezlenebilmeleri, çeşitli kimyasal grupların yapıya katılmasıyla spesifik etki oluşturmaları gibi avantajlara sahiptir.
Bu çalışmada peptidler için küresel ve lineer yapılı polimerik taşıyıcılar incelenmiştir, taşıyıcı polimerlere çeşitli örnekler verilmiş, sistemlerin immün yanıta etkileri ve avantajları incelenmiştir.
Anahtar Sözcükler: Polimerik taşıyıcılar, polielektrolitler, nanopartiküller.

1. INTRODUCTION

Vaccines provide an effective protection against infectious diseases by stimulating the immune system of animals and humans. Vaccination has begun at IIXXth century with Edward Jenner's discovery of smallpox vaccine and with recent developments of molecular biology,

* Corresponding Author/Sorumlu Yazar: e-mail/e-ileti: zmustafaeva@yahoo.com, tel: (212) 383 46 30

immunology and biotechnology has allowed the development of vaccines with increased effectiveness, reliability and quality. Synthetic peptides and recombinant DNA technology have particularly opened a new page in modern medicine with the usage of subunit vaccines. The synthetic vaccines rely on the principle of injection of antigenic epitope which produces specific immune response rather than introduction of the whole microorganism [1,2,3,4]. However; low solubility, instability, low antigenic properties due to molecular size, non-specific and non-biocompatibility properties and lacking systematic toxicity has restricted their functionality. In order to get ahead of these setbacks, synthetic peptides were introduced to organism with the help of polymeric carriers [4]. Studies have shown there are multiple benefits of binding various molecules to polymeric carriers;

- ✓ Increased solubility of therapeutic drugs with low solubility
- ✓ Peptides are especially fragile, polymers protect molecules from hydrolysis and enzymatic degradation by forming a micro-environment
- ✓ By modifying the properties of the carrier such as solubility, controlled drug release can be achieved.
- ✓ The drugs can be carried to the targeted tissue; this is particularly useful for drugs that are toxic for the rest of the tissues.
- ✓ They can harbour multiple vaccine components[5,6].

Polymeric carriers can be obtained by using synthetic or natural polymers. Using natural and synthetic polymers have advantages and disadvantages compared to each other (Table 1) [7]. Natural polymeric carriers are usually focused on proteins and polysaccharides and have significant properties. Some of these are; they are the naturally occurring polymers and produced in living organisms, meaning they can be obtained easily with non-expansive methods. In recent years, tendency towards synthetic polymers rather than natural polymers has risen. The most significant advantages of synthetic polymers over natural polymers are; predictable uniformity and obtaining products with high purity [8]

Table 1. Advantages and disadvantages carrier molecules obtained from synthetic and natural polymers [7].

Natural Polymers		Synthetic Polymers
• Low toxicity • Biocompatibility • Biodegradability	Advantages	• Obtaining product with desired structure and molecular weight • Biocompatibility • Obtaining product with high purity
• Complex structures • Cost and difficulty of extraction process • Difficulty of purification process	Disadvantages	• Toxicity

Various synthetic polymer carrier systems have been developed to peptide transfer [Figure 1].

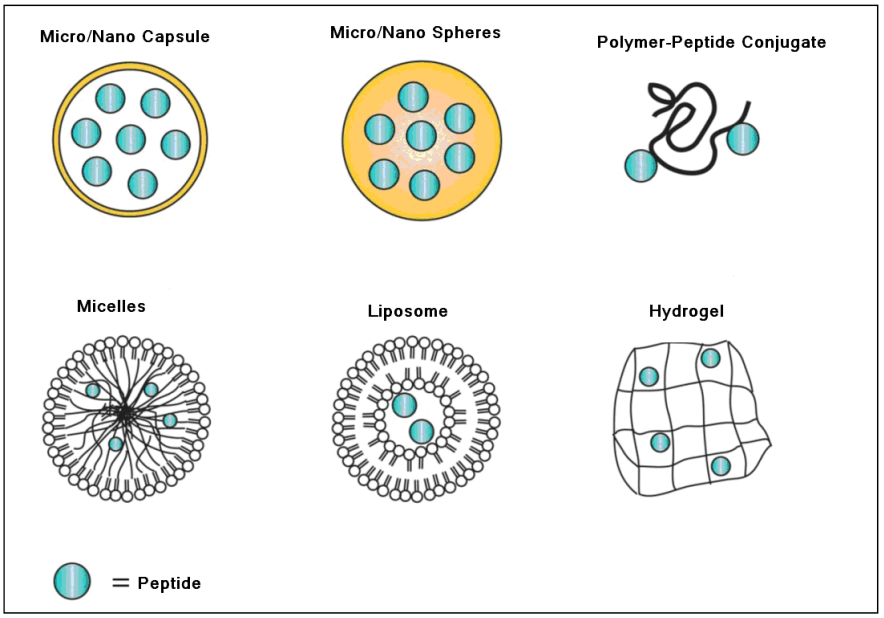

Figure 1. Polymeric carrier system used for transfer of peptide based vaccines [9].

Polymeric carriers used;

2. SPHERICAL STRUCTURED CARRIERS

2.1. Nanoparticles

Polymeric nanoparticles are colloidal systems with a size varying between 10-1000 nm [10]. Nanoparticles can be separated as "nanospheres" and "nanocapsules" according to their preparation methodology. Nanospheres are the systems in which the active ingredient is dissolved, dispersed or partially adsorbed to the matrix structure whereas nanocapsules are the vesicular systems in which the active ingredient is surrounded by a polymeric membrane with an aqueous or fatty nucleus. They are also known as reservoir systems (Figure 2). [11]

There have been various polymers and co-polymers used at designing nanoparticles. Especially biodegradable synthetic polymers have been the centre of attention because of their application in controlled/ continuous drug release and their high biocompatible properties. Because of their advantages polymeric nanoparticles have been effective carriers for vaccines containing bioactive molecules, peptides, proteins and nucleic acids. Aside these functions, nanoparticles can be used as immunostimulants by being carried to antigen producing cells to produce immune response [10]. Studies that has used nanoparticles as adjuvants have had the best results were obtained with particle sizes varying between 100-200 nm.'s it was also recorded that the adjuvant affect decreases as the size of the particle increases. It was recorded that adjuvant effect of influenza vaccine increases when it is used with poly(acrylic) nanoparticles compared to classical adjuvants. Mucosal response was also generated by targeting nanoparticles [12,13].

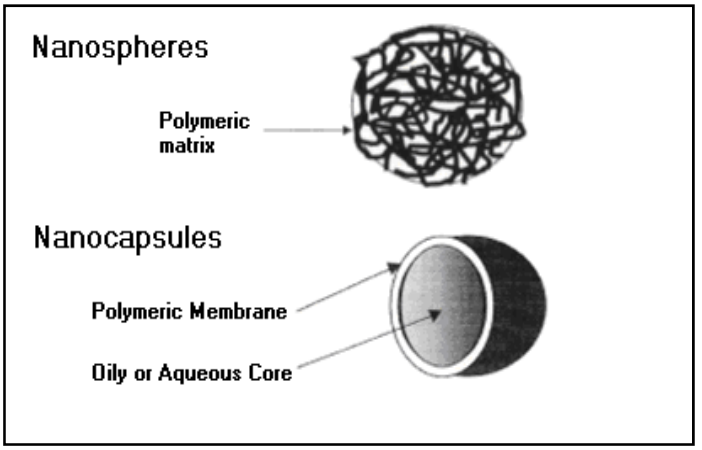

Figure 2. Structure of nanospheres and nanocapsules [7].

Types of nanoparticles;

- **Synthetic Polyanhydrides** is an interesting antigen carrier polymer because of its bioadhesive properties, specific immune system cell interactions and low toxicity. Poly (methyl vinyl ether-co-maleic acid) (PVM/MA) is a good example for polyanhydrides. In the studies done by Gòmez and colleagues the behaviour of PVM/MA nanoparticles as intradermal vaccine carriers using ovalbumin as a model peptide [14]. It was observed that OVA-charged nanoparticles produce a stronger humoral response compared to ovalbumin [10,15]. Anhydrites containing hydrophobic branch and oligomeric ethylene glycol have been a new hope for next generation of vaccines with their immunomodulatory properties [10].

- **Polysaccharide structured** molecules such as inulin, algin, hyaluronic acid has gathered the attention of scientist as antigen carriers. Chitosan has been especially studied broadly for antigen carrier potential. Chitosan which is obtained by partially deacetylation of chitin has biodegradable, mucosa-adhesive and immunity regulatory properties which makes it a good candidate for a vaccine carrier. There are numerous reports stating that chitin's anti-tumour activity and cytokine producing macrophage stimulation which enhances immunity to non-specific bacterial, viral and infective agents [15,16].

These nanoparticles are formed by jellification of chitosan with ionotropic anions, their size vary between 150-450 nm.'s and they are adapted for carrying significant amounts of antigen [15].
Vaccine applications done with tetanus toxoids loaded chitosan carriers showed higher antibody levels compared to soluble antibodies and these levels reaching 10 times of the initial value within 24 weeks [15].

- **Polyamide structured** molecules such as poly(□benzyl L-glutamate) (PBLG), a synthetic polypeptide, can work as multidirectional carrier (Figure 3). Most significant advantage of this polymer is, diverse chemicals can partake in its structure which can produce specific co-polymers [17]. PBLG was used as polymer-peptide block nanoparticle with subunit influenza vaccine in order to increase its effectivity, its chain length and functionality gave the carrier multidirectional property. Nanoparticles observed in mice models showed a significant increase in IgG serum aside a strong T2 response and relatively weak T1 response. In order to increase T1 response an immunomodulatory (CpG) was encapsulated [17-19].

- **Polyester structured nanoparticles** Polyesters are that contain unstable aliphatic ester bonds thermoplastic polymers. Their biodegradable and biocompatible properties have made

these polymers alluring for vaccine and drug carrier agents. In recent years, PLA and PLGA (d, l-lactic-co-glycolic acid) have been the most popular polymers used in nanoparticle production because of their advantageous properties [10].

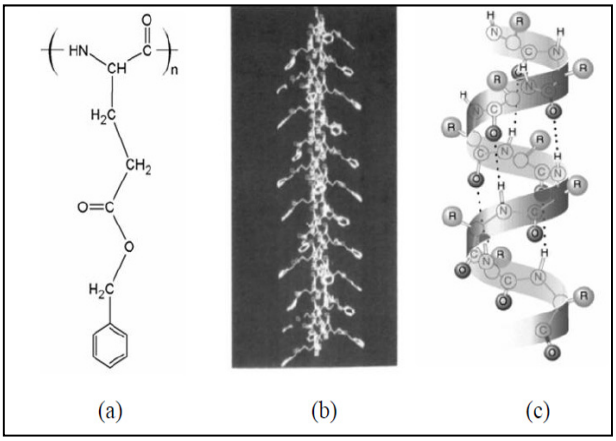

Figure 3. PBLG structure [17].

PLGA and its monomers, polylactic acid (PLA) and polyglycolic acid (PGA) have been approved by FDA for clinical trials because of their biocompatible and biodegradable properties (Figure 4) [20]. By changing the ratio of these monomers, PLGA co-polymer can be synthesized with varying molecular weight, physical, chemical and physiochemical properties according to will. PLGA inside the body fluid is first degraded into PLA and PGA, it is then metabolized inside the citric acid cycle where it is converted into carbon dioxide and water [10,15,21].

This nanoparticle is insufficient when it comes to stability because of its bulk degradation mechanism which disrupts the molecular firmness with charged complex. It also has low mucosal tissue penetration [10]. There have been numerous strategies to overcome these obstacles, the most common being covering PLGA nanoparticles with PEG (poly ethylene glycol). PEG is a hydrophilic, neutral and biocompatible polymer that can be added combined with PLGA via surface adsorption, covalent bonding or during nanoparticle formation. Studies done have shown that nanoparticles covered with PEG showed improvement in drug delivery at nasal mucosa. Studies done with Hepatitis B surface antigens showed that PLGA nanoparticles covered with PEG produced a faster immune response [15,22]. Furthermore recent studies have demonstrated nanoparticle loading method effects immune response. PLGA encapsulated method has a hihger efficiciancy in lysosomal escape and cross-presentation of antigen from dendritic cells compared to antigen absorbed on nanoparticle method [23].

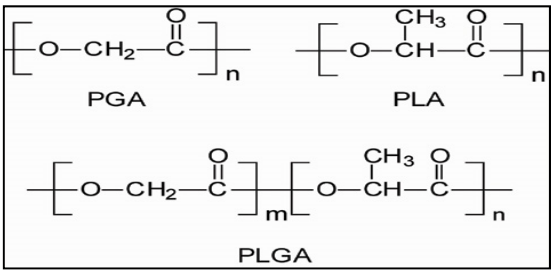

Figure 4. Structure of PLA PGA and co-polymer PLGA [36]

Poly ε- caprolactone (PCL) has a non-toxic, relatively non-expensive properties which makes it popular in controlled drug release systems. Its non-crystalline structure and hydrophobic property makes it harder to degrade compared to PLGA (Figure 5) [24]. It is suitable for drug release systems that are planned to be used over a period of a year [10].

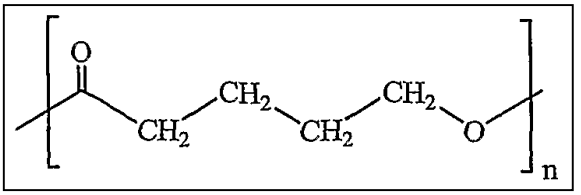

Figure 5. Structure of PCL [22]

2.2. Micelles

Micelles are spherical structured particles composed out of amphiphilic blocks. Studies have shown that the shape of micelles can also be rods, vesical, lamella, hexagonal or hollow spheres. Micelle forming pharmaceutical surfactants has low molecular weight and they also increase the solubility of substances.

Studies done on polymeric micelles are focused on di-block co-polymer structure. Most of the amphiphilic co-polymers used in drug release contain a derivative of polyester, polyether or poly (amino acid). Usually a hydrophobic core containing biodegradable polymer such as poly (-caprolactone), poly (d, 1-lactic acid) or poly (B-benzoyl-L-aspartate) protects the partially soluble effective ingredient from aqueous environment. Hydrophilic polymer that constitutes the outer shell of polymeric micelle is responsible from interactions with plasma proteins and cell membranes along with effective steric protection. Poly (ethylene glycol), poly hydroxyethyl methacrylate, poly (N-vinyl- pyrrolidine) and poly (vinyl alcohol) are the most common hydrophilic blocks used.

The driving forces of micelle formation are; electrostatic interactions between block co-polymers such as poly (ethylene glycol)-poly (L-lysine) and poly (Ala)-poly (Sar), polyanions and polypeptides, or intramolecular hydrogen bond forces forming core-shell-corona structure [6,26,27].

2.3. Polymeric Carries with Liposome Properties

Liposomes are spherical colloidal systems composing out of an aqueous core and lipophilic void between lipid bi-layers. Mixing phospholipids with water forms suspended lipid multi-layers which form concentric dual layers containing an aqueous phase in between. During the enclosing of vesical, which was formed during the development of dual layers, water soluble molecules such as drugs, peptide, protein, nucleic acid are entrapped [28].

Liposomes can be formed out of natural or synthetic materials [29]. They have a molecular size varying between 60-150 nm.'s and their synthetic peptide with drug or antigenic feature carriage capacity is high.

Liposomes ability to dissolve both hydrophilic and hydrophobic substances makes it an efficient carrier for simultaneous transfer of both types of molecules (Figure 6).

Studies done with liposome carried antigens showed higher levels of antibodies compared to free antigens, this information allowed further investigation adjuvant properties of liposomes to take place [6,28,29].

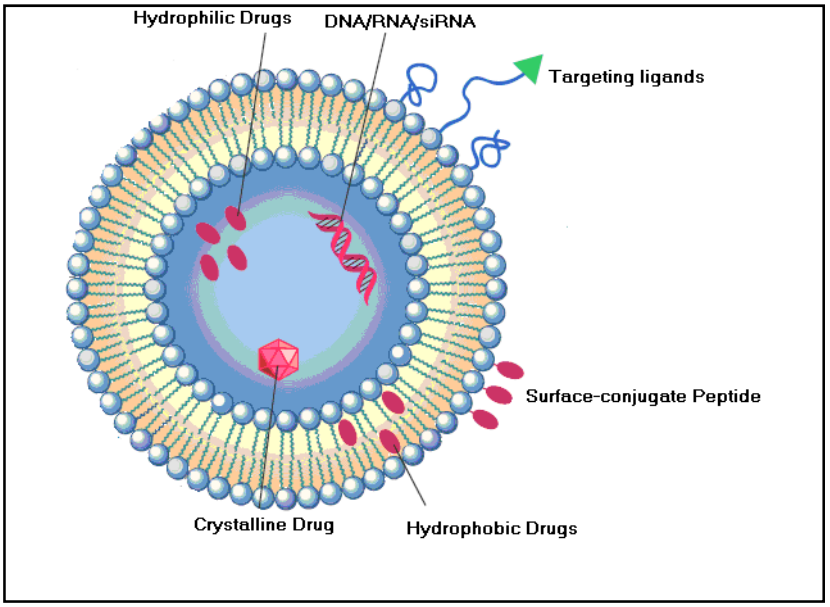

Figure 6. Lipid bi-layer and liposome structure that can carry both hydrophobic and hydrophilic molecules simultaneously [29]

When using liposomes, problems with binding of drug, stability of drug, lipid layer structure and leakage of entrapped molecules inside the biological systems can arise. Non-specific drug leakage and instability of liposome-drug conjugate increases toxicity, decreases uptake duration and it may even cause the therapeutic efficiency to perish. Drug leakage and liposome-drug conjugate stability is closely related with lipid layer fluidity. On a physicochemical stand point; long hydrocarbon chains and un-saturated chains with few side branches can form firm lipid layers with decreased leakage and improved stability. Instability of liposomes can be naturally decreased by choosing long, linear and saturated lipids and polymerized lipids [10].

3. LINEAR STRUCTURED CARRIERS

Synthetic peptides have a few disadvantages such as small molecular size, low antigenicity, etc. allowing it to bind linear such as polyacrylicacid, poly-N-isopropylacrylamide, poly-N-vinyl pyrrolidine, polyvinylpyridine-polysetylpyridine and polyvinylpyrrolidine-polyacrylicacid, polyoxidonium [30].

Polyelectrolytes containing positive or negative charged groups are used to form a conjugate with peptides. These electrolyte groups dissociate to their ions and charge the polymer. Polyelectrolytes can show the properties of both polymers and salts and that's why they can also be called polysalts [31,32]. Their solution, like salts, conduct electricity and their structure like polymers is viscous. Their solubility depends on the electrostatic relation between the water and charged polymer branches. Most of them are in polyelectrolyte form as in most of biomolecules [31].

Polyelectrolytes can be classified according to their source (natural/synthetic/chemically modified) and according to their structure (homoelectrolytes and co-polyelectrolytes). Polyampholytes contain both anionic and cationic groups which makes it polyelectrolyte. Polyelectrolytes can be linear or crosslinked (Table 2) [31,32].

Table 2. Classification of polyelectrolytes [31].

Name	Category (based on the charge type)
Natural Polyelectrolytes	
Nucleic acids	Polyanion
Poly (L-lysine)	Polycation
Poly (L-glutamic acid)	Polyanion
Carrageenan	Polyanion
Alginates	Polyanion
Hyaluronic acid	Polyanion
Chemically modified biopolymers	
Pectin	Polyanion
Chitosan (deacetylation of chitin)	Polycation
Cellulose - based	Polyanion or polycation
Starch - based	Polyanion or polycation
Dextran - based	Polyanion or polycation
Synthetic polyelectrolytes	
Poly (vinylbenzyl trialkyl ammonium)	Polycation
Poly (4-vinyl-N-alkyl-pyridimiun)	Polycation
Poly (acryloyl-oxyalkyl-trialkyl ammonium)	Polycation
Poly (acryamidoalkyl-trialkyl ammonium)	Polycation
Poly (diallydimethyl-ammonium)	Polycation
Poly (styrenesulfonic acid)	Polyanion
Poly (vinylsulfonic acid0	Polyanion
Poly (acrylic or methacrylic acid)	Polyanion
Poly (itaconic acid)	Polyanion
Maleic acid/ diallyamine copolymer	Polyampholytic

There have been many studies done on immunogenicity on polyelectrolytes. Injecting sheep erythrocytes, which is a classic adjuvant, with polyelectrolytes (polycarbonacids, polycarbonbases and their copolymers) showed a significant increase in immune response. The fact that polyelectrolytes increase immune response and give long term immunity against pathogens like bacteria, viruses, etc. gave the idea of using these to make synthetic vaccines to Mustafev and colleagues. When using uncharged polymers which that cannot be charged in aqueous environment it was observed that there was no increase in immune response, meaning it shows no adjuvant effect [32-34].

The ease of synthesis and modification of polyelectrolytes, the ability of producing polyelectrolyte with varying molecular weight, net electrical charge, conformation are some of the advantages polyelectrolytes. They are water soluble and can be synthesised to form numerous complexes. Molecular weight of polyelectrolytes is proportional to polymerization degree. Increasing the polymerization degree affects the complex formation in a positive way. Aside this electrochemical properties of polyelectrolyte are usually dependant on poly-ion chain length, meaning properties such as precipitation depend on polymerization degree [33,34].

Some of the linear polymers used as carrier peptides;

- **Polyacrylic Acid (PAA)**

Polyacrylic acid is a weak electrolyte that is widely used in biomedical applications such as immunologic, enzyme immobilization and controlled drug release. Carboxylic acid groups on PAA allow the biomolecule to bind to a polymer in soft chemical conditions without causing any modifications on the biomolecule [35].

In many studies it was shown that polyacrylic acid acts as an effective adjuvant for strong primary and secondary immune response [36,37].

Polyacrylic acid can form inter-polymer complexes with various proton acceptor, non-ionic polymers and their derivatives and cationic polyelectrolytes in aqueous environments or in organic solvents. Nature of the polymer, molecular weight of the polymer, the solvent, pH, ionic strength of solvent, temperature and polymer concentration are some of the parameters affecting complex formation [38].

- **N-Vinyl Pyrrolidine-co-Acrylic Acid (VPAA) Copolymer**

Studies done with PAA have shown strong immunogenic affect however their toxicity has prevented widespread usage of the polymer. To get ahead of this problem N-Vinyl Pyrrolidine-co-Acrylic Acid (VP-AA) copolymer was synthesised (Figure 7). In studies done with varying molecular weights and ratios, it was shown that VPAA has a strong immunogenic affect and have a low toxicity thanks to its N-vinyl pyrrolidine group [39,40]. Amide group present in NVP, gives the molecule a high binding affinity to large and small molecules, it is a good H acceptor which allows it to form co-polymer with a lot of monomers [41]. Low chemical toxicity, high solubility in water and organic, the ability to form complexes with various substrates such as dyes, polymers, surfactants has allowed this polymer to be used in different applications such as hydrogel drug release membranes [42]. The synthesised polyanions is an ideal carrier for peptides due to its negatively charged groups which reacts with oppositely charged group of the peptide [39]. Pharmaceutical studies done with VPAA showed that the carboxylic acid group of the polymer has antimicrobial properties [41].

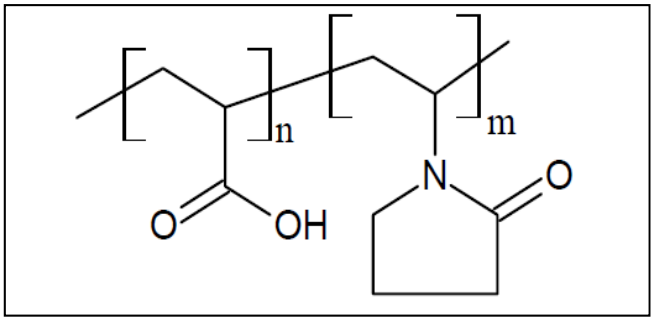

Figure 7. Presentation of N-Vinyl Pyrrolidine-co-Acrylic Acid (VPAA) Copolymer [42]

- **Polyoxidonium (POX)**

This polymer is a cationic synthetic polyelectrolyte and it is known that it has high adjuvant activity [36]. Polyoxidonium is a triple co-polymer composed out of; 1,4-ethylenepiperazine, 1,4-ethylenepiperazine-N-oxide and (N-carboxymethylene)- 1,4-ethylenepiperaziniumbromide and has a molecular weight varying between 60-100 kDa (Figure 8). N-oxide groups optimal concentration in its structure is critical to minimize toxicity and keep the immunostimulant activity at desired levels. N-oxide groups can degrade under temperature which makes this co-polymer degradable *in vivo* making it possible releasing small fragments of the polymer [36,43].

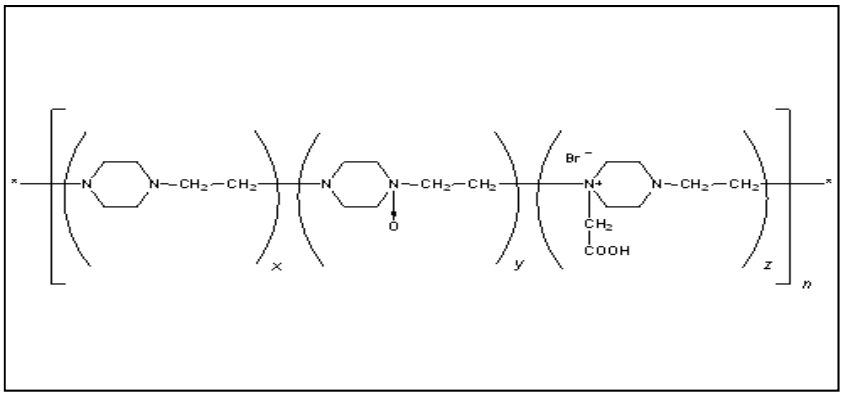

Figure 8. Structure of Polyoxidonium (POX) [44]

The first clinical application of polyoxidonium was observed in influenza vaccine, it was conjugated with hemagglutinin and neuraminidase antigenic compounds and positive results were obtained. Clinical application with weakened measles, mumps and rubella vaccines can also be found [43].

- **Poly (N-isopropylacrylamide) [Poly (NIPAAm)]**

This linear and environmentally friendly smart polymer is a popular because it can dissolve reversibly until it is heated in organic solvents at atmospheric pressure and room temperatures which is unlike most of the other polymers. This is why it is one of the most widely studied synthetic polymers as biomaterials. The transformation of Poly (NIPPAm) from hydrophilic to hydrophobic form happens abruptly and the temperature where this transformation takes place is also known as lower critical solution temperature (LCST). For this polymer, LCST was determined experimentally as between 29-32°C. Because P(NIPAAm) is a temperature sensitive polymer; under the LCST point (32°C) the polymer is water soluble. Above this temperature; the polymer releases the water molecules attach to it and becomes hydrophobic making it participate (Figure 9). This behaviour is reversible and takes place in a short interval (1–2°C). LCST behaviour of P(NIPAAm) is used for affinity separation, immunity testing and forming protein-peptide conjugates [45,46].

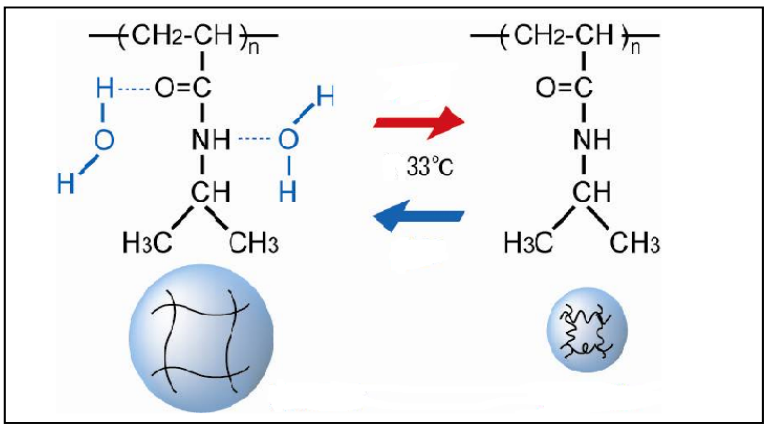

Figure 9. Structural alteration mechanism of P(NIPAAm) at LCTS temperature [47].

4. RESULTS AND DISCUSSION

Binding of macromolecules to peptides is widely used in medicine and biotechnology; in drug release systems, in immobilized enzymes, at immunoassay studies, vaccine synthesis, etc. Polymeric carriers are especially studied in vaccine and drug technology, next generation vaccines can use antigenic peptides for targeting which allow humoral and cellular immune response. Polymeric carriers are also important to increase the biocompatibility of the peptide, control the release in biological systems and make an effective vaccine prototype.

Acknowledgement / Teşekkür

This work is supported by Office of Scientific Research Project Coordination in Yıldız Technical University project ((Project Name: Development Of New Generation Peptide Vaccine Models Against Ebola Virus Disease; Project Number: 2015-07-04-KAP01).

REFERENCES / KAYNAKLAR

[1] Akbuğa J., "Farmasötik Biyoteknoloji Ürünleri", [Internet], Available from;http://www.e-kutuphane.teb.org.tr/pdf/mised/mayis02/10.pdf, [accessed 05.09.2015].

[2] Büyüktanır Ö.,"Günümüzde Biyoteknolojik Bakteriyel Aşılar", Atatürk Üniversitesi Vet. Bil. Derg.5 (2): 97-105, 2010.

[3] Altınsoy N., "Aşı Üretim Teknikleri ve Kontrolü", A.Ü. Veteriner Fakültesi, Mikrobiyoloji Anabilim Dalı**,** Ankara, 2007.

[4] Skwarczynski M., Toth I., "Peptide-based synthetic vaccines", Chemical Science, 7:2-842-854, 2016.

[5] Kahraman G., "Bor nötron yakalama terapisi için bor içeren polimerik taşıyıcı tasarımı ve hücre kültürlerinde kullanımları", Hacettepe Üniversitesi FBE Kimya Mühendisliği Anabilimdalı Yükssek lisans tezi, 2004.

[6] Varypataki E.M., et.al., "Synthetic long peptide-based vaccine formulations for induction of cell mediated immunity: A comparative study of cationic liposomes and PLGA nanoparticles", Journal of Controlled Release, 226: 98–106, 2016.

[7] [Internet] Available from; "http://www.diss.fuberlin.de/diss/servlets/MCRFileNodeServlet/FUDISS_derivate_0000 00001958/1_Chapter1Introduction.pdf?hosts=" [accessed 15.05.2015].

[8] Muhamad I., et.al., "Designing Polymeric Nanoparticles for Targeted Drug Delivery System", Nanomedicine, 287.

[9] Janssen M., et.al., Drugs and Polymers for Delivery Systems in OA Joints: Clinical Needs and Opportunities, *Polymers*, *6*(3), 799-819, 2014.

[10] Rebouças J., Esparza I., et.al., "Nanoparticulate Adjuvants and Delivery Systems for Allergen Immunotherapy", Journal of Biomedicine and Biotechnology,10.1155/2012/474605, 2012.

[11] Şengel-Türk T., Hasçicek C., "Polimerik Nanopartiküler İlaç Taşıyıcı Sistemlerde Yüzey Modifikasyonu", Ankara Ecz. Fak. Derg., 38 (2) 137-154, 2009.

[12] Öner F., "Nanopartiküller:Kolloidal Katı İlaç Taşıyıcı Sistemler", FABAD 8:250-260, 1983.

[13] Fattal E., "Nanoparticle and liposomes for drug targetting", Berlin, pp 1-4, 1997.

[14] S. Gomez C. Gamazo, B. S. Roman, M. Ferrer, M. L. Sanz, and J.M. Irache, "Gantrez_AN nanoparticles as an adjuvant for oral immunotherapy with allergens," *Vaccine*, vol. 25, no. 29, pp. 5263–5271, 2007.

[15] Correia-Pintoa J.F., Csaba N., Alonso M. J., "Vaccine delivery carriers: Insights and future perspective", International Journal of Pharmaceutics 440 (2013) 27– 38, 2012.
[16] Powell S.B., Andrianov A.K., Fusco P.C., "Polyionic vaccine adjuvants: another look at aluminum salts and polyelectrolytes", Clin Exp Vaccine Res, 4:23-45, 2015.
[17] Özcan İ., "Effects of sterilization techniques on the PEGylated poly (γ-benzyl-L glutamate) (PBLG) nanoparticles", Acta Pharmaceutica Sciencia 51: 211-218, 2009.
[18] Christophe Q.,"Adjuvanted nanoparticulate seasonal influenza vaccines", Leiden University, 2013.
[19] Yu D., et.el., "PEG-PBLG Nanoparticle-mediated HSV-TK/GCV Gene Therapy for Oral Squamous cell Carcinoma", Nanomedicine 3(6):813-821, 2008.
[20] Makadia K.H., Steven J.S., "Poly Lactic-*co*-Glycolic Acid (PLGA) as Biodegradable Controlled Drug Delivery Carrier", Polymers (Basel). 2011 Sep 1; 3(3): 1377–1397, 2011.
[21] Derman S., Kızılbey K., Akderste Z., "Polymeric nanoparticle", Sigma 31:107-120, 2013.
[22] Bharali D.J., Pradhan V., Elkin G. et al., "Novel nanoparticles for the delivery of recombinant hepatitis B vaccine," Nanomedicine, vol. 4, no. 4, pp. 311–317, 2008.
[23] Liu L., et. al., "Immune responses to vaccines delivered by encapsulation into and/or adsorption onto cationic lipid-PLGA hybrid nanoparticles", Journal of Controlled Release, S0168-3659(16)30045, 2016.
[24] Mi L., "Ultrasound-activated anti-infective coatings and devices made thereof", WO2006019848 A1, 2006.
[25] Başbağ A., "Development of Alternatıve Polymerıc Carrıers for Cyclosporıne a Release and İnvestıgatıon of *ın vıtro* Release Kinetics", Hacettepe Üniversitesi Kimya Mühendisliği Anabilim, Yüksek Lisans Tezi, 2010.
[26] Sezgin Z., Yüksel N., Baykara T., **"**İlaç taşıyıcı sistemler olarak polimerik misellerin hazırlanması ve karakterizasyonu", Ankara Ecz. Fak. Derg, 32(2) 125-142.
[27] Kahraman, E., "Polimerik miseller ve nazal yol ile uygulanmaları", İstanbul Üniversitesi Eczacılık Fakültesi Dergisi, 41, 2010.
[28] Mansuroğlu B., "Biyohibrid yapılı ve biyotaklitçi polimer-peptid konjugatlarının geliştirilmesi", Yıldız Teknik Üniversitesi Fen Bilimleri Enstitüsü Biyomühendislik Anabilim Dalı, Doktora Tezi, 2007.
[29] Çağdaş M., Sezer A., Bucak S., "Liposomes as Potential Drug Carrier Systems for Drug Delivery", 978-953-51-1628-8, 2014.
[30] Gurhan Deliloğlu S.I., Akdeste M.M., Akdeste Z.M., Aynagoz G., Unver G., Unal N., Celik N.,"Preparation of synthetic peptide FMDV vaccine with newly developed antigen-polymere conjugates be used as immunogen and vaccine in veterinary medicine", In:Report of the Session of the Research Group of the Standing Technical Committee of the European Commission for the Control of Foot-and-Mouth Disease, Appendix41, pp.349-357, FAO, 2002.
[31] Lankalapalli S., Kolapalli V.R.M., "Polyelectroyte complex",Indian J Pham Sci"., 71(5):481-487, 2009.
[32] Mustafaev M.I., "Biyopolimerler", TÜBİTAK Marmara Arastırma Merkezi (MAM) Matbaası, Gebze, Kocaeli, 1996.
[33] Mustafaev M., Yucel F., Cirakoglu B., Bermek E., "Immune response to progesterone involved in Cu2+-mediated polyanion-protein complex-antigen specificity and affinity of hybridoma clones", Immunology Letters, 52 (2-3): 63-68, 1996.
[34] Mustafaev M., "Polyelctrolytes in İmmunology: Fundamentals and Perspective", Tr. J. of Chemistry 20:126-138, 1995.

[35] Topuzogulları M., Koç Çakır R., et.al., "Conjugation, characterization and toxicity of lipophosphoglycan-polyacrylic acid conjugate for vaccination against leishmaniasis", Journal of Biomedical Science, 2013.

[36] Kabanov V.A., "From synthetic polyelectrolytes to polymersubunit vaccines", *Pure Appl. Chem.*, Vol. 76, No. 9, pp. 1659–1677, 2004.

[37] Mansuroğlu B., Mustafaeva A. Z., "Characterization of water-soluble conjugates of polyacrylic acid and antigenic peptide of FMDV by size exclusion chromatography with quadruple detection", Materials Science and Engineering, 112–118, 2012.

[38] Karahan M., "Metal İçeren Fonksiyonel Biyopolimer Sistemlerin Geliştirilmesi", Yıldız Teknik Üniversitesi FBE Kimya Anabilim Dalı Biyokimya Anabilim Dalı, 2009.

[39] Petrov R.V., Khaitov R.M., Zhdanovt V.M., Sinyakov M.S., Norimov A.Sh., Nekrasov A.V., Podchernyaevat R.Y., Kharitonenkovi I.G., ve Shchipanovat M.V., "Influenza Virus Antigens Conjugated with A Synthetic Polyelectrolyte: A Novel Model of Vaccines, Vaccine, Vol. 3, 393, 1985.

[40] Uelzmann H., "Copolymers of Acrylic Acid and N-Vinylpyrrolidone-2" Journal of Polymer Science,XXXIII:377-379, 1958

[41] Hemalatha P., et.al., "Antibacterial Properties of Poly(N-Vinylpyrrolidone-co-Acrylic Acid)/ Diethylaminoethanol Ester", Indian Journal of Advances in Chemical Science 2:50-54, 2014.

[42] Derman S., Kadriye K., Mansuroglu B., Mustafaeva Z., "Poly(N-Vinyl-2-Pyrrolidone-co-Acrylic Acid)- Bovine Serum Albumin Complex Formation Studied by HPLC and UV/Vis Spectroscopy", Romanian Biotechnological Letters Vol.17, No.4, 2011.

[43] Synpol, [Internet] "Polyoxidonium", Available from: http://www.drugfuture.com/chemdata/polyoxidonium.html, [accessed 02.10.2015].

[44] "PLGA-PEG Block Copolymers for Drug Formulations", [Internet], Available from; http://www.drug-dev.com/Main/Back-Issues/PLGAPEG-Block-Copolymers-for-Drug-Formulations-406.aspx, [accessed 22.09.2015].

[45] Oskay E., "Hücre Kültüründe Tümör Hücrelerine Akıllı PolimerikTasıyıcılar ile Antisense Oligonükleotit Aktarımı", Hacettepe Üniversitesi Fen Bilimleri Enstitüsü Biyomühendislik Anabilimdalı Yüksek Lisans Tezi, 2005.

[46] Almeida H.,et.al., "Temperature and pH stimuli-responsive polymers and their applications in controlled and selfregulated drug delivery", Journal of Applied Pharmaceutical Science 02 (06); 2012: 01-10, 2012.

[47] [Internet] Available from: "http://www.tuat.ac.jp/~tokuyama/research.html" [accessed 20.05.2015].