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## REVIEW

Application of nanoparticles drug delivery systems  
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## ABSTRACT

Despite significant progresses in the development and emergence of new antibiotics, it is still a major problem to treat intracellular bacterial pathogens in human infections. Concentration in specific doses of antibiotics is often dependent on the type of treatment and some drugs may be prescribed in large doses for more efficiency. This is important not only economically, but also may cause localized or side effects in individual patients. On the other hand the emergence of antimicrobial resistance caused that treatment of inner cellular bacteria has not been successfully completed. The use of nanoparticles for drug delivery systems caused to optimize the effectiveness of the treatment process and subsequently better in this kind of infections. polymeric nanoparticles are of particular importance due to biodegradability and biocompatibility. On the other hand, the nanoparticles particularly increase the phagocytic activity and operate as desired in drug delivery systems. Due to great efforts in recent years in the field, there is no doubt that the nanoparticles improve the treatment of intracellular bacterial agents, optimize the chronic infections and minimize their side effects. In this review, applications of drug delivery systems are used, leading to effective performance on target bacteria.

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**Key words:** Brucellosis - Drug delivery systems - Dendrimers - Nanoparticles - Liposom - Listeriosis - Quantum dots - Tuberculosis.

Today, the chemotherapy of bacterial infections remaining in cells is considered as a serious challenge in treating this type of disease. In other words, several bacteria inhibit the function of bactericidal agents by creating silent infection in cells. So in this case, the cells are not only incapable of removing bacteria intracellularly, but also may act as reservoirs of infection and spread to other cells and organs. As a result, most intracellular bacteria are safe from immune system or antibiotics. Although, many antibiotics are effective against bacteria, they are ineffective in penetrating into the cells containing bacteria.<sup>1</sup>

In other words, the treatment of intracellular infections with antibiotics is not always successful and mul-

tidrug administration is more effective than single drug administration, but it is more difficult for patients, leading to patients' low satisfaction and due to the long-term use, they finally stop to take antibiotic. Considering these factors, there is also the possibility of recurrence after treatment. Drug delivery systems can detect target bacteria and places, enhance the capabilities of drug therapies and ultimately prevent the problems associated with high absorption of antibiotics.

In general, pathogenic bacteria are divided into three categories in terms of the invasion of eukaryotic cells: 1) extracellular bacteria; 2) facultative intracellular bacteria; 3) obligate intracellular bacteria.<sup>2,3</sup>

Intracellular bacteria are those that are capable of

surviving and growing inside the cell and are usually placed within the macrophages of host cell. As mentioned above, intracellular bacteria are divided into two facultative and obligate categories, facultative intracellular bacteria include *Legionella*, *Rickettsia*, *Mycobacterium tuberculosis*, *Listeria monocytogenes*, *Salmonella* and obligate intracellular bacteria are *Mycobacterium leprae* and *Coxiella burnetii*.<sup>4-6</sup>

It should be noted about the importance of treating intracellular pathogens that *Mycobacterium tuberculosis* is one of the factors causing the death of more than 3 million people annually in the world. It is estimated that one-third of the world's population are infected with *Mycobacterium tuberculosis*, which is the causative agent of *Tuberculosis*. On the other hand, human brucellosis disease is still discussed as a major problem in today's world with the death of more than 500 thousand people per year. So it is needed to take necessary measures for early and timely treatment of such diseases to treat these pathogens. As said, long-term use of antibiotics has been associated with side effects, sometimes severe. Therefore, it seems necessary to use new nanotechnology tools for effective and efficient treatment. In this review article, some basic terms are defined and at the end, we investigate some nanosystems used in the treatment of intracellular bacteria and studies conducted.

It was firstly performed by Japanese scientist named Professor Takeru Higuchi. His first paper on the application of physicochemical properties for drug delivery and control was published in 1961, with the title of "an Introduction to drug delivery and design".<sup>7, 8</sup> His classic equation was founded on the use of porous systems in 1963. According to this theory, drug delivery system founded and continued between the years 1960-1985 and many drugs were absorbed from metabolic routes, particularly orally and mucosally. It was determined in the future that the rate of drug delivery was a ratio of the inverse square of time.<sup>9, 10</sup>

In general, systems which control the drug-release time and space are called drug-delivery systems. A method, by which the drug is delivered to the body, has a significant effect on the efficiency of treatment. Some of the drugs have an optimum concentration range, that maximum efficacy of drug is obtained in this area and concentrations of higher and lower than this threshold are toxic or has no therapeutic effect. Additionally, to

success in the treatment of severe disease such as cancer, there is an urgent need to a targeted drug delivery system with ability to release the drug in the target organ. To achieve this purpose, some studies have been conducted on the control of pharmacokinetics, pharmacodynamics, non-specific toxicity, immunogenicity and efficacy of drugs. These new strategies are often called drug delivery systems which are based on a combination and solidarity among polymer science, pharmaceuticals, chemistry of bioconjugates, molecular biology and genetic engineering.<sup>11</sup>

### Types of carriers used in drug delivery systems

#### Liposomes

Liposomes had been considered as one of the largest studies in the field of drug carriers since early 1690, the year it was discovered. They possess a spherical structure or several central lipid layers, usually composed of phospholipids and cholesterol and have a hydrophilic end. Anyway, they have a low toxicity and immunogenicity because of their resemblance to the structure of cell membranes. Liposomes are useful, since they have adaptive structures to trap drugs with different properties. Depending on the method of preparation, they can be created as different sizes and different layers. Based on these parameters, liposomes can be divided into three categories: small unilamellar vesicles (SUL), large unilamellar vesicles (LUV) and small plurilamellar vesicles (SPLV). Anyway, liposomes can trap the drug by hydrophilic and hydrophobic combinations. Water-soluble compounds are placed in aqueous areas, while soluble lipid is connected to lipid membrane. When liposomes are absorbed intravenously, they are rapidly removed by phagocytic cells, especially by the macrophages in the liver (liver Kupffer cells) and spleen. This is one of the reasons leading to the study on the applications of liposomes in the treatment of intracellular infections.<sup>12</sup>

#### Polymeric nano- and microparticles

Polymeric nano- and microparticles are used as alternative systems for liposomes that it is effective in solving the problems of stability and absorption in biological fluids. Optimal stability and the ability to control encapsulated drug delivery are of the main advantages of polymeric nanoparticles to liposomes. Followed by

the necessary improvements in microencapsulation techniques and development of new polymer, some of these vectors became special issues for some categories such as hormones, anticancers, antigens and antibodies.<sup>13</sup> In general, polymers used are divided into two groups: natural and synthetic polymers. Natural polymers including human and bovine serum, gelatin, collagen, alginate, chitosan, hyaluronan and starch derived from natural sources. Among synthetic polymers, most polymers used are PLGA and PLA.<sup>14</sup> Both are non-

toxic, biodegradable and biocompatible and are also approved by Food and Drug Administration for human consumption.<sup>15, 16</sup> In Table I<sup>17-34</sup> are listed some of the applications of polymeric nanoparticles. In 2016 Amini *et al.* using chitosan nanoparticles containing antigens ESAT-6 increased induction of interferon gamma and IL-4 and IgG compared with the control group. The results showed that chitosan nanoparticles caused suitable induction of immune responses against *Tuberculosis* disease.<sup>26</sup> Chitosan nanoparticles containing curcumin

TABLE I.—Polymeric nanoparticles designed for microorganisms drug delivery.

Formulation	Drug	Targeted microorganism	Action	References
PLGA	Gentamicin doxycycline	<i>Brucella melitensis</i>	Improved efficacy	17
Poly lactic-co-glycolic acid (PLGA) Nanoparticles	Phosphorothioate antisense oligonucleotide	HIV	Defense of oligonucleotides from poverty	18
Poly (isohexylcyanoacrylate) (PIHCA) nanospheres	Primaquine and ampicillin	<i>Leishmania donovani</i> , <i>Salmonella typhimurium</i> and <i>Listeria monocytogenes</i>	Particle itself Exhibitions Antimicrobial action	19, 20
Polyethylene glycol (PEG)-PLA nanocapsule	Halofantrine	<i>Plasmodium berghe</i>	Lengthy flow half-life	21
Chitosan dextran sulfate	Ciprofloxacin Ceftriaxone	<i>Salmonella</i>	Efficient to drug delivery	22
Poloxamer 188 coated poly(epsilon-caprolactone) (PCL) nanosphere	Amphotericin B.	<i>Candida albicans</i>	Lesser <i>in vivo</i> toxicity due to reduced addition in kidney and liver	23
Chitosan	Gentamicin (AOT)	<i>Staphylococcus aureus</i> & <i>Listeria monocytogenes</i>	1) High cellular accumulation 2) Improved efficacy against bacteria used	24
Alginate nanoparticle	Rifampicin, isoniazid, pyrazinamide, and ethambutol	<i>Mycobacterium tuberculosis</i>	1) High drug cargo 2) Enhanced pharmacokinetic 3) High beneficial efficacy	25
Glycosylated polyacrylate nanoparticle	Beta-lactam/ciprofloxacin	<i>Staphylococcus aureus</i> and <i>Bacillus anthracis</i>	1) Better bioavailability 2) Higher beneficial efficiency	26, 27
Poly-lactide-co-glycolide (PLG) nanoparticle	Rifampicin, isoniazid, pyrazinamide and ethambutol	<i>Mycobacterium tuberculosis</i>	1) Improved bioavailability 2) Improved pharmacodynamic	28
Poly(ethylene oxide) (PEO)-modified poly(epsilon-caprolactone) (PCL) nanoparticle	Saquinavir	HIV	1) Protect the drug from cytochrome C metabolism 2) Avoid P-gp efflux pump	29
Poly (D,L-lactide) (PLA) nanospheres	Arjunoglucoside	<i>Leishmania donovani</i>	Condensed toxicity	30
Magnetite block ionomer complexes	Gentamicin	<i>Brucella melitensis</i>	Increase efficiency in the treatment of mice with samples	31
Solid lipid nanoparticles (SLN)	Rifabutin	<i>Mycobacterium tuberculosis</i>	SLN, were new potential vehicles for pulmonary delivery of antitubercular drugs.	32
Stable therapeutic nanocarriers (NCs)	Rifampicin	<i>Mycobacterium tuberculosis</i>	NC form resulted in significantly improved activity compared to that of the free drug against intracellular <i>M. tuberculosis</i>	33
Chitosan-chondroitin sulfate	Amphotericin B	<i>Leishmania donovani</i>	Chitosan-chondroitin sulfate has improved the toxicity and compared to traditional way of its direct administration	34

had been treat *Staphylococcus aureus* and *Pseudomonas in vivo* was reported.

In this study, nanoparticles containing curcumin significantly inhibits the growth of bacteria *Staphylococcus aureus* and *Pseudomonas*.<sup>35</sup> The immunogenicity amount of omp19 antigens by the chitosan nanoparticles in *Brucella melitensis* and *Brucella abortus* was performed by Abkar *et al.* In this study, they proved that antigen was produced as a form of nanoparticles can be induced Th1, Th2 in the laboratory sample. On the other hand oral administration of antigen will induce Th1 and Th17. The results indicated that the use of these antigens as a form of nanoparticles is to induce immune responses and protection against *Fors melitensis* and *abortus*.<sup>36</sup>

Chitosan nanoparticles were produced as a vaccines against the *E. coli O157H7* by Doavi *et al.* In this study, they concluded that the oral absorption of the nanoparticles reduced significantly the bacterial infection in the mouse model.<sup>37</sup>

In using nanofibers, chitosan-containing nanoparticle vaccine Ipad antigen of *Shigella spp.* was performed and checked it in pig model issue by Jhantygh *et al.* In this study, they concluded that the absorption of inhaled nanoparticles is induce immune responses in guinea pig models. It also became clear that chitosan nanofibers able to transfer Ipad antigen in nasal mucosa.<sup>38</sup>

Platinum oxide nanoparticles inhibition of the growth of bacteria *Lactobacillus* and *Pseudomonas stutzeri* was reported by Rezai-Zarchi *et al.* These results showed that carbohydrates can serve as a carrier for platinum oxide nanoparticles, and nanocomposites can have potential biological applications.<sup>39</sup> In 2012, Zand *et al.* revealed that the surface-area-to-volume ratio (SA:V) of nanoparticles plays an impressive role in sporecide properties.<sup>40</sup>

In 2015, Georgi Khah *et al.* provided an overview paper on the use of nanotechnology in biomedicine. They have introduced the employment of nanostructures in order to identify and treat pathogens. Mentioning the lab on chip theory, they focused on portability and ease of use of diagnostic and therapeutic methods.<sup>41</sup>

In 2015, Ahmadi-Aghk *et al.* used nanofibers, leading to healing of skin lesions. They concluded in this article that nanofiber scaffolds can perform delivery tools for drug and growth factor.<sup>42</sup>

### Silica nanoparticles

These nanoparticles have many applications in biological systems such as drug delivery, imaging and oxygen carriers. Their use in antibiotic delivery is proven. Biocompatibility and non-toxicity are their obvious features, depending upon the dose and route of absorption in the body. The use of these nano-particles made it possible to diagnose and treat enterotoxin C produced by *Staphylococcus aureus*. Anyway, their biocompatibility led to their use in drug delivery system as means and carriers appropriate to reduce the cytotoxicity and side effects and reduce the dosage of antibiotics.<sup>43</sup>

### Solid-lipid nanoparticles

They are named as colloidal carriers in the size of 50 nm to 1  $\mu$ , composed of lipid structures spread in water or surfactant solution. In the past decade, solid-lipid nanoparticles (SLN) were more involved in the drug/delivery systems. They are used in the incorporation of hydrophilic and lipophilic drugs in the lipid substrate. They are prepared from solid-lipid substrate at room temperature in less than one micron in size. Some of the benefits include the ability to control the release of nanoparticles in drug delivery, to increase drug stability and drug loading, the possibility of incorporating lipophilic drugs and non-biototoxicity of the carrier. The nanoparticles have been used in ciprofloxacin drug delivery to treat infections, as well as in the treatment of *Mycobacterium tuberculosis*. In total, SLN nanoparticles have a high potential for antibiotic carriers compared with liposomes and polymeric nanoparticles.<sup>44</sup>

### Quantum dot

Quantum dots are nanocrystals formed by semiconductor material, which has interesting physical properties including high quantum efficiency, light resistance and regular optical luminescence. These factors caused them to act as a powerful tool in biological applications. They have small structures with abundant capabilities and 1-10 nm in size. Water-soluble quantum dots may crosslink with some materials such as antibiotics, oligonucleotides and small molecules. Quantum dots loaded by antibodies against the target bacteria have been used in the treatment of *E.coli O157*.<sup>45</sup>

## Dendrimers

Dendrimers are a group of macromolecules made for planned and targeted delivery of drugs. They are branched spherical units with different-sized polymeric branches and possess a particle size distribution of unit and monodisperse spherical symmetrical shape.<sup>46</sup> The other feature of dendrimers is the presence of suitable empty spaces within them that can be appropriate sites for the placement of the types of hydrophobic and hydrophilic drug molecules. Also, the presence of various well-known functional groups on their surface converts them to carriers appropriate for the connection of the types of drug molecules or different ligands and it can help in targeting drugs by this way.<sup>47</sup> Many factors are involved in the transfer of nanoparticles from digestive system to bloodstream. Generally, drug-carrying nanoparticle are being collected in the digestive system from intestine by structures called Peyer's patches and cells called M-cell and after transferring to lymph vessels, they enter blood vessels. Many bacteria have been treated with PAMAM dendrimer and nadifloxacin.<sup>48</sup> Also, a type of dendrimers (PEGylated copolymers of lysine) is used in the treatment of plasmodium falciparum.<sup>49</sup>

### The treatment of bacterial factors associated with drug delivery systems:

#### Tuberculosis

From the perspective of epidemiology, Asia and Africa are faced with the highest annual occurrence. However, the disease acts as an opportunist by reducing immune deficiency syndrome. Some of the main drugs obtained against the agent, acted as encapsulated, leading to reduced toxicity and ultimately reduced continuous dose in this way. The first treatment using a drug delivery system was to obtain long-term therapeutic levels of drug in the infection sites. In this case, significant progress has been achieved. When a contractual formulation was recorded, rifampicin and isoniazid were determined by low concentrations in serum and measured in blood circulation cycle less than 24 hours. On the contrary, intravascular absorption of 12 mg/kg and 10 mg/kg isoniazid and rifampicin-encapsulated liposomes led to long-term loading in the level of blood serum and organs about 5 to 7 days, respectively.<sup>50</sup> However, the effect of chemotherapy on isoniazid and rifampicin-encapsulated liposomes

was showed in tuberculosis murine models as once a week. It was found that only one-third dose of isoniazid and rifampicin was necessary for treatment.

At the same doses, antibiotics loaded with liposomes markedly reduced the number of bacteria in the lungs. Besides the use of these liposomes, no sign of liver toxicity was observed by them. Therefore, the use of liposomes in treating tuberculosis may lead to the emergence of a hypothesis for appropriate treatment consistent with the patient's condition, with low prices and reduced frequent dose and toxic effects. Encapsulation of antituberculin drugs in other polymeric particles is considered as a strategy to optimize the disease treatment. In the past few years, several antituberculin drugs including PLA and PLGA microparticles and mainly nanoparticles have been studied broadly. One of the most recent projects for tuberculosis chemotherapy is the inhalational use of antimycobacterium drug carriers directly into the lungs. In this case, the use of high doses of drug in the site of infection results in reduced toxicity at the site of infection. Medicinal kinetics and antimycobacterial effects of polymeric particles of drugs in pigs have been studied in Guinea. Polymeric carriers have shown a better drug kinetics than liposomes. In a study conducted on pigs in Guinea, the use of a single dose of rifampicin, isoniazid, pyrazinamide encapsulated in PLGA led the drug to be remained stable in blood plasma and lungs from 6 to 8 days and finally, to 11 days. Some researchers showed that PLGA microparticles could release isoniazid, rifampicin and pyrazinamide to 9 days in different organs and finally to 3 days in plasma. In this case, the weekly oral absorption of nanoparticles showed that similar results are obtained to achieve a daily intake compared to free drug. However, the effect of chemotherapy on PLGA encapsulated drugs was prescribed after oral administration as subcutaneous injection, but with better and easier intake advantages. The use of nanoparticles absorbed after 10 days in the tissues of mice infected with *Mycobacterium tuberculosis* completely removed this infection.<sup>51</sup> In 2011, Saraogi *et al.* tried to treat tuberculosis with gelatin and isoniazid, which effective results were obtained in this study.<sup>52</sup>

#### Brucellosis

While this endemic disease is limited in some areas of the Mediterranean and the advanced Asian coun-

tries, with the increase in international tourism in Africa and Latin America, the possibility of the disease has increased in non-endemic areas. Brucellosis is a systemic disease that can enter in any organ or tissue. Onset of the disease begins with fever, sweat, fatigue, malaise and joint pain, and even more insidiously, it may take from a week to a month. Most relapses occur in the first six months of disease after treatment. The main treatment of human brucellosis is to overcome the symptoms of the disease and prevent the recurrent of the disease. However, when it is very difficult to completely remove the microorganisms, and it is required to combine synergistic antibiotics for long-term patient treatment satisfaction.

Although, many tests have been good and effective in antibiotic treatment of this factor in the past two decades. More appropriate antimicrobial therapies for human brucellosis have continued to be controversial. In total, doxycycline oral combination and intramuscular injection of aminoglycosides with the recurrence of 5% are considered to be the most effective treatment.<sup>53</sup> The commonest recurrence is the need for non-intestinal absorption and the potential for the toxicity of aminoglycosides. Aminoglycosides effective role in this combination should be emphasized. When aminoglycosides are used individually, their effect is reduced, resulting from the low role of the antibiotics within the cell. They showed inherent synergy with tetracycline in both *in vitro* and *in vivo*.<sup>54</sup> The oral combination of doxycycline and rifampin for 45 days is the best alternative treatment so far.

Prior *et al.* produced PLGA 502H microparticles by drying method. They investigated nanoparticles as gentamicin-containing capsules in laboratory conditions, leading to effectively reduced bacteria.<sup>55</sup> On the other hand, unfortunately nanoparticles accumulated in the body as they did not have a good therapeutic effect, causing the lack of a good cycle in target organs such as the liver.

Accumulation of particles was performed using solvent evaporation method. In fact, Lecaroz *et al.* showed that when 502H or 752H micro- or nanoparticles were absorbed, gentamicin was successfully transferred into the liver and spleen.<sup>56, 57</sup> Drug kinetic parameters showed that the distribution of gentamicin-loaded PLGA is different if compared to the free drug. Higher concentrations of antibiotics in the liver and spleen were observed when these loaded particles had been absorbed. Genta-

micin durability is related to the half-life of nanoparticles and the nature of aminoglycosides. These drugs are very stable and are not metabolized in the liver; as a result, their intracellular maintenance is very high. Moreover, when mice were chronically infected with virulence strains, infection level was markedly decreased in the spleen with two formulations of microparticles compared to free gentamicin that was not effective. Studies showed that after two weeks only 752H microparticles remained in contact with the spleen and in low quantities in the liver. However, gentamicin was found in the liver and spleen for four weeks after absorption. This concentration was determined 7 days. Polymeric nanoparticles prepared by PEO-PtBA and carrying streptomycin and doxycycline act as an alternative to treat brucellosis.<sup>58</sup> Using the nanoparticles, mice were infected with *B. melitensis* and treated with 9 mg/kg streptomycin and 1.8 mg/kg doxycycline. Both drugs and nanoparticles showed reduced infection in the spleen after 3 days, 0.51 and 0.79, respectively, but only nanoparticles obtained in the liver had 0.79% reduction. Therefore, in recent years the use of alternative methods in drug delivery systems using aminoglycosides has shown to be effective in the treatment of human brucellosis. In 2013, Imbuluzqueta *et al.* attempted to treat *Brucella melitensis* in a mouse sample by modified PLGA and loading gentamicin. They reached the effective reduction of 2 units on a logarithmic scale in laboratory conditions in media culture with the use of THP-1 monocytes. They studied 3.23 log reduction and complete elimination of 50% of the full *Brucella* infection in mice in-vivo using mentioned nanoparticles. In conclusion it can be said that the use of modified PLGA polymeric nanoparticles will be as a promise for the treatment of brucellosis in humans.<sup>17</sup>

### Salmonellosis

*Salmonella* is one of the major food poisoning diseases affecting human beings. It may be raised as the most important pandemic zoonosis disease under natural conditions. *Salmonella* is a facultative intracellular pathogen which can cause gastroenteritis, enteric fever, bacteremia and local chronic infection. More recently, drug resistance against fluoroquinolones is the most important problem in treating *Salmonella* that is resulted from the creation of betalactamase and increase in drug resistant strains.

Several studies have been conducted with use of antibiotic loading to measure and the competence of the effects of these vectors in laboratory models. PIHCA nanoparticles had interesting results, so that they reduced mortality in mice with acute infection by *Salmonella typhimurium*.<sup>19</sup> All mice remained 60 days after inoculation of infected bacteria using a single dose of 0.8 mg encapsulated ampicillin. In comparison, 3 doses of 32 mg of the free ampicillin were used to obtain the same effect. All mice untreated and treated with drug-free nanoparticles died after 10 days of infection. This increased effect was attributed to the selected target of carriers with infected tissue observed *in vitro*.<sup>59, 60</sup> In other words, the measurement of polyalkylcyanoacrylate nanoparticles was effective against non-divided bacteria. Page-Clisson and colleagues studied the effects of these carriers on models infected by *Salmonella typhimurium*.<sup>61</sup> They found that although there are bactericidal properties in the early stage of infection, when bacteria are divided, neither of ciprofloxacin or ampicillin encapsulated drug can markedly lead to infection reduction in the liver and spleen in the later steps. Therefore, the key role of bacterial metabolism on sensitive to antibiotics was reappraised.

Ampicillin entrapped in liposomes was used to treat *Salmonella*. Using nanoparticles, ampicillin covered with liposomes was accumulated mainly in the spleen and less in the liver. However, liposomes were effective for the acute treatment of *Salmonella typhimurium* less than PIHCA nanoparticles.<sup>62</sup> The use of a single dose of 0.8-mg ampicillin containing liposomes resulted in hindering the development of the disease up to 60%. However, ampicillin nanoparticles were more effective for the liver than liposomes. Apart from ampicillin, aminoglycosides loaded with liposomes have been used in the *in vitro* treatment of *Salmonella*. Twenty mg of streptomycin doses loaded into the liposomes remained in mice for one month. When 2 doses of 20 mg or 80 mg single dose was absorbed, the mice survived for 55 days.<sup>63</sup> However, streptomycin located inside the liposome reduced toxicity. Gentamicin loaded by liposomes acted more effective against *Salmonella typhimurium* and *Salmonella dublin*. Ten mg of labeled gentamicin led to survival of mice infected with *Salmonella typhimurium* regardless of liposomes absorbed a few days after infection.<sup>64</sup> While mice tolerate *Salmonella dublin* infection, absorption of 2, 10 and 20 mg of gentamicin

loaded inside the liposome leads to the survival of mice with 80%, 90% and 100%, respectively.<sup>65</sup> PH sensitive liposomes have been used to treat mice infected with *Salmonella typhimurium*.<sup>66</sup> When gentamicin was injected into the liposome sensitive to PH, its concentration reached 153- and 437-fold in the liver and spleen, respectively. However, the concentration of antibiotics in the kidneys decreased 2.8 times. The desirable behavior of medicinal kinetics optimized the effect of carriers in body condition. Absorption of 0.2 to 5 mg/kg antibiotics loaded in liposomes resulted in a decrease of 3 units of bacteria in the spleen, while the same doses were ineffective compared to the free drug. Therefore, alternative methods such as drug delivery systems have a support role at high levels and bactericidal activity should be used as a treatment for infected *Salmonella* in the future. In 2013 Gnanadhas *et al.* reached desired results in the treatment of mice infected with *salmonella* using chitosan-dextran sulfate nanoparticles and ciprofloxacin.<sup>22</sup>

### Listeriosis

Listeriosis is a food poisoning disease caused by *Listeria monocytogenes* facultative intracellular pathogen which is one of the most important causes of risky infections, including sepsis and meningoencephalitis, especially in infants during parturition, in the elderly and people with compromised immune. Direct treatment for listeriosis includes the combination of ampicillin and aminoglycosides, that gentamicin is usually used.<sup>67</sup> Wherein the bacteria are left, ampicillin different vectors have improved in order to increase the intracellular capabilities. Studies on uninfected mice showed that when ampicillin absorbed intravenously via coated liposomes, its concentration went up quickly in the liver and mainly in the spleen. However, the effect of liposomes and free antibiotic in mice infected with *Listeria monocytogenes* was measured. Seven days after treatment, ampicillin loaded in liposomes reduced 3.2 units in the liver and 2.8 units in the spleen, but free ampicillin was ineffective. In another study, Bakker-Woudenberg *et al.* observed that high concentration of ampicillin, when it was loaded into the liposomes, has been achieved in the liver.<sup>68</sup> However, they found that the lipid composition of carriers has been effective on the behavior of liposomes in body conditions. For ex-

ample, although maintained liposomes are less movable, they remain in the liver more time; they kill bacteria in the fluid less than movable liposomes. About nanoparticles, polymeric nanoparticles coated with ampicillin were more effective than free ampicillin in a listeriosis chronic test in a non-thymic group in mice.<sup>69</sup> Seven days after treatment, ampicillin nanoparticles were completely removed from the liver, while free ampicillin in the liver was not removed completely during 19 days after the last test even with high doses of 48 mg. Regardless of the spleen, the difference between treatments was not very clear, but nanoparticles loaded with ampicillin led to decrease in infection compared with the control group. In 2012 Imbuluzqueta *et al.* attempted to treat infected mice using modified PLGA nanoparticles. they had resulted that usage of proper nanoparticle containing antibiotics led to improve efficacy against of *listeria monocytogenes* and *S.aureus* with compared of free drugs.<sup>24</sup>

### Conclusions

No antibiotic treatment for the eventual elimination of intracellular bacteria such as *Brucella* and *Mycobacterium* has been done so far. On the other hand, it seems necessary to take some measures for combining antibiotics in order to reduce complications of the disease and its relapse. Accordingly, pharmaceutical scientists conducted some studies on using a variety of nanoscaled carriers to increase the ability to absorb the drug and reduce the dose consumed. On the other hand, side effects are also reduced with this method.

The use of drug delivery systems plays an important role in the management of intracellular infections, because the use of mentioned method leads to formulations appropriate for antimicrobials and increases their pharmacological effects and reduces the cost of treatment.<sup>70</sup> Some advantages of drug delivery systems are the ability to maintain the drug in a relatively constant amount in a specified time period, the ability to adjust the rate of drug releasing, the possibility to deliver the drug to the specified issue and the ability to deliver several drugs with one formulation.

Among other nanoparticles, polymeric nanoparticles are of particular importance due to biodegradability and biocompatibility. On the other hand, the nanoparticles particularly increase the phagocytic activity and oper-

ate as desired in drug delivery systems. Due to great efforts in recent years in the field, there is no doubt that the nanoparticles improve the treatment of intracellular bacterial agents, optimize the chronic infections and minimize their side effects. The new generation nanoparticles may also act purposefully and possess some ligands such as antibodies, peptides and aptamer.

At present, the application of nanotechnology in drug delivery system is broadly expanded. We will observe in the future that it will change industries dependent on biotechnology and pharmaceutical. Methods for targeted treatment of pathogens in nanotechnology research centers will play a prominent role in the future and can be helpful for the treatment of intracellular bacterial agents. The proper use of pharmaceutical nanotechnology sciences promises new treatments in the future on the basis of lower costs and greater productivity. On the other hand, the proper use of pharmaceutical nanotechnology will perhaps also enhance the general health of the population and eventually public health.

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