



# Asian Journal of **Biochemistry**

ISSN 1815-9923



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## Application and Safety of Erythrocytes as a Novel Drug Delivery System

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### ABSTRACT

Carrier erythrocytes are one of biological drug delivery systems have been investigated in recent years. They are biocompatible, biodegradable as well as they have long life span. This review deals with criterion, requirement and methods of drug loading as well as characterizations, safety and application of erythrocyte as drug delivery system. The drugs with high toxicity, such as cardiotoxic and neurotoxic are selected to be encapsulated in the erythrocytes because of their potential for delivery to the liver, spleen and lung. Physical methods like endocytosis and osmotic based methods are used for drug loading into erythrocytes, as well chemical methods are used for the same purpose. After loading of therapeutic agent on erythrocytes, the carrier cells are exposed to physical, cellular as well as biological characterizations. The resealed erythrocytes were used either for sustained release or targeted release of therapeutic agents. The targeted resealed erythrocytes are structurally modified either by glutaraldehyde, oxidizing agents or by drug itself. The modified carrier erythrocytes have tendency to be phagocytosed, therefore, they removed from the circulation and targeted to the organs of reticuloendothelial system.

**Key words:** Erythrocytes, carrier erythrocytes, drug delivery, drug targeting

### INTRODUCTION

The drug delivery systems currently available enlist carriers that are simple soluble macromolecules such as monoclonal antibodies, soluble synthetic polymers, polysaccharides in addition to biodegradable polymers. Moreover they include complex multi-component structures like microcapsules, microparticles, lipoproteins, liposomes, ghost cells and cells (Pierige *et al.*, 2008).

The cellular carriers have been a useful device as drug delivery system, these carriers, including, leukocytes, platelets, hepatocytes, fibroblasts and erythrocytes (Hamidi and Tazerzadeh, 2003; Rossi *et al.*, 2005). Erythrocytes have many advantages over the other cellular carriers in its selectivity to deliver the bioactive agents to any organs. The destruction of modified erythrocytes occurs in liver, spleen and lymph nodes; therefore, they are used as carriers to deliver the drugs to Reticulo Endothelial System (RES) (Gopal *et al.*, 2007). In addition, the possibility of targeting carrier erythrocytes to non-RES organs has been exploited. Also these cells are

non-immunogenic and biodegradable; they freely circulate throughout the body and offer ease of preparation. Furthermore, they have the capacity to carry large amounts of drug Hamidi and Tajerzadeh (2003). Carrier erythrocytes can be used as circulating drug reservoirs within the circulation (Jain and Jain, 1997, 1998).

### **ERYTHROCYTES MORPHOLOGY**

The normal erythrocyte is a biconcave, ellipsoidal disc with depressions located in the center on both sides. The average erythrocyte is 8.6  $\mu\text{m}$  in diameter and 1.9  $\mu\text{m}$  in thickness and has a mean volume and surface area of 86 and 145  $\mu\text{m}^2$ , respectively (Patel, 2009). The blood volume of a normal adult human male is about 7% of body weight and about 6.5% in a female. For the blood volume of 5 L in a 70 g man, erythrocytes make up about 40 to 50% of this volume and there are about  $5 \times 10^{12}$  erythrocytes per liter of the blood (Magnani *et al.*, 2002).

The red blood cell membrane is dynamic, semi-permeable components of the cell, associated with energy metabolism in the maintenance of the permeability characteristic of the cell of various cations ( $\text{Na}^+$ ,  $\text{K}^+$ ) and anions ( $\text{Cl}^-$ ,  $\text{HCO}_3^-$ ) (Patel, 2009).

Each RBC contains about 280 million hemoglobin molecules. A hemoglobin molecule consists of a protein called globin, composed of four polypeptide chains; a ring like non-protein pigment called a heme, is bound to each of the four chains. At the center of the heme ring combine reversibly with one oxygen molecule, allowing each hemoglobin molecule to bind four oxygen molecules. RBCs include water (63%), lipids (0.5), glucose (0.8%), mineral (0.7%), non-hemoglobin protein (0.9%), methemoglobin (0.5%) and hemoglobin (33.67%) (Gupta *et al.*, 2010).

### **CRITERION FOR USE OF ERYTHROCYTES AS DRUG DELIVERY SYSTEM**

The normal physiology of erythrocytes gives opportunity to use them as drug delivery system. The main function of erythrocytes is the transport of  $\text{O}_2$  from the lungs to tissues and the  $\text{CO}_2$  produced in tissues back to lungs. Thus, erythrocytes are a highly specialized carrier systems in the body due to the following criterion (Gopal *et al.*, 2007):

- The elastic, biconcave shape enables erythrocytes to squeeze through narrow capillaries
- Erythrocytes make up about 40 to 50% of blood volume therefore; a large amount of substance can be encapsulated in erythrocytes
- Mature erythrocytes are simple in structure; they have neither nucleus nor other organelles, for that reason some of the intracellular space exists for drug transport
- Life-span of RBCs is 100-120 days in the circulation before removing so can be used for sustained delivery of therapeutic agents
- Erythrocytes are selectively removed from circulation by the macrophages in the Reticulo Endothelial System (RES), hence be able to used in targeting of drugs to RES
- The breakdown products are recycled; hemoglobin is break down into globin and hem. Globin degraded to amino acids for amino acid pools in the body, while iron reused in hemoglobin synthesis

### **DISADVANTAGES OF ERYTHROCYTES AS DRUG DELIVERY SYSTEMS**

The modifications that occurred during loading procedure of the drugs into the erythrocytes accelerate their removal by the RES *in vivo* (Papadatou *et al.*, 2009). Also certain encapsulated substances may be leaked from the loaded erythrocytes (Gupta *et al.*, 2010). Add to this the storage problem that need for conditioning carrier cells (Hirlekar *et al.*, 2008). Otherwise contamination is

possible due to the origin of the blood, the equipment used and the loading environment. Special precautions are required for the collection and handling of the erythrocytes (Hamidi *et al.*, 2007b; Rossi *et al.*, 2006).

### NECESSITIES FOR DRUGS ENCAPSULATION INTO ERYTHROCYTES

The molecules should be polar, hydrophilic, resist degradation within erythrocytes, lack of physical or chemical interaction with erythrocyte membrane and well defined pharmacokinetic and pharmacodynamic properties (Gupta *et al.*, 2010; Patel, 2009). Non-polar and hydrophobic molecules may be entrapped in erythrocyte in their respective salts. Molecules which interact with the membrane and cause deleterious effects on membrane structure are not considered to be appropriate for encapsulation in erythrocyte. Erythrocytes can entrap a wide variety of biologically active substance (5000-600,000 Daltons in size) (Hamidi *et al.*, 2007b).

### APPLICATIONS OF RESEALED ERYTHROCYTES

Resealed erythrocytes have been proposed as delivery systems for a variety of applications in human and animal's medicine. In vivo application of the drugs loaded erythrocytes are used either for prolonged drug released or for drug targeting to RES or non RES.

**Targeting of bioactive agents to RES:** Damaged erythrocytes are rapidly cleared from circulation by phagocytic cells in liver and spleen. Targeting of the drug decreases its side effects and the dose to be administered as well as drug utilization (Balamuralidhara *et al.*, 2011). Modifications of erythrocytes membranes accelerate their targeting to the liver as well as spleen. The treatment of the carrier erythrocytes with certain substances gives rise to alterations in the properties of the loaded erythrocytes. These substances include antibodies, glutaraldehyde, sialic acid and sulfhydryl containing substances (Gupta *et al.*, 2010).

- **Glutaraldehyde:** The treatment of loaded erythrocytes with glutaraldehyde enhances their properties as carrier systems. It has been observed that the erythrocytes treated in this way are more stable which increases their osmotic resistance, as well as their resistance to turbulences. It means that the output of the encapsulated substance from these erythrocytes into the circulatory flow is reduced (Talwar and Jain, 1992). Similarly, the treatment with glutaraldehyde increases the selectivity of the erythrocytes towards the RES and specifically, towards certain organs such as the liver and the spleen (Millan *et al.*, 2004b)
- **Ascorbate and ferrous ions:** The chemical alteration of the erythrocyte membrane with substances as ascorbate/ $\text{Fe}^{+2}$ , diamide or band 3-cross-linking reagents can induce increased the uptake of modified red cells by macrophages (Millan *et al.*, 2004a)
- **Biotin:** The surface modification of erythrocytes has also been addressed using phenylhydrazine and N-hydroxysuccinimide ester of biotin (NHS-biotin) which increase the macrophage uptake of loaded erythrocytes both *in vitro* as *in vivo* (Mishra and Jain, 2002). Moreover, biotinylation of erythrocytes may also be a way of preparing immuno-erythrocytes attached to biotinylated antibodies that are stable in circulation and capable of recognizing antigens (Gupta *et al.*, 2010)
- **Antibody:** Coating the loaded erythrocytes by anti-Rh or other types of antibodies is another method that makes the erythrocytes more recognizable by RES macrophages. In this

technique targeting of erythrocytes is either spleen or liver. If the antibody used as ligand in form of immunoglobulin G, targeting to spleen is preferred; while if used in form of immunoglobulin M type, the liver targeting is dominant (Erchler *et al.*, 1986)

- **Other means of modification:** Pre-exposing the carrier erythrocytes to thermal shock increase the up taking of loaded erythrocytes by RES (Ihler *et al.*, 1973). Also oxidant compounds like azodicarboxylic acid bis (dimethylamide) increases the uptake of loaded erythrocytes by RES (Arias *et al.*, 2010), they are reactive toward the sulfhydryl group-containing proteins of the cell membrane (Ihler *et al.*, 1973). The enzyme neuraminidase as well as the proteolytic enzymes, also has been exploited to improve RES targeting of carrier erythrocytes with some degree of success (Millan *et al.*, 2004b)

**Targeting to sites other than RES-rich Organs:** Erythrocytes loaded with drugs have the ability to deliver a drug or enzyme to the macrophage-rich organs, Also, such cells have been used to target organs outside the RES. Co-encapsulation of paramagnetic particles, photosensitive agents in erythrocytes along with the drug to be targeted; application of ultrasound waves as well as site-specific antibody attachment to erythrocyte membrane (Hamidi *et al.*, 2007b). The magnetic erythrocytes, resulting from the co-encapsulation of the drugs with some ferrous fluids such as cobalt-ferrite and magnetite, have been reported to direct the encapsulated drug predominantly to the desired sites of the body by means of an external magnetic field. The magnetically guided erythrocytes have been tested successfully for targeting anti-inflammatory drugs to inflamed tissues (Markov *et al.*, 2010; Ross, 2009). Photosensitized erythrocytes have been studied as a photo-triggered carrier and delivery system for methotrexate in cancer treatment. Moreover, carrier erythrocytes fused to the thermo-responsive liposomes and their localization using the external thermal source (Hamidi *et al.*, 2007b).

**Carrier erythrocytes as slow drug release system:** Slow release dosage forms are designed to obtain a prolonged therapeutic effect by continuously releasing medication over an extended period of time after administration of single dose (Hossain *et al.*, 2004).

Carrier erythrocytes have long life span in the circulation, so that they can be used as circulating depots for antitumor, antiparasitics, antibiotics as well as cardiovascular drugs. This happened only when the drug and the selected method for the drug loading don't change the morphological and physiological parameters of erythrocytes (Gupta *et al.*, 2010). Various bioactive agents encapsulated in erythrocytes are developed for the sustained release in circulation to allow effective treatment of diseases. Resealed erythrocytes serve as an ideal carrier for antineoplastic agents, antimicrobial drugs, vitamins and steroids (Gupta *et al.*, 2010).

**Erythrocytes as circulating bioreactors:** Erythrocytes have been realized as carriers for enzymes to serve as circulating bioreactors. Sometimes it is desirable to decrease the level of circulating metabolites that can enter erythrocytes. Erythrocytes have also been used as circulating bioreactors for the controlled delivery of antiviral drugs (Magnani and DeLoach, 1992).

## **METHODS OF DRUG LOADING INTO ERYTHROCYTES**

Erythrocytes can be isolated from blood using a suitable anti coagulant (Hamidi *et al.*, 2007b). Different sources such as human (Harisa *et al.*, 2011), rats (Mishra and Jain, 2002),

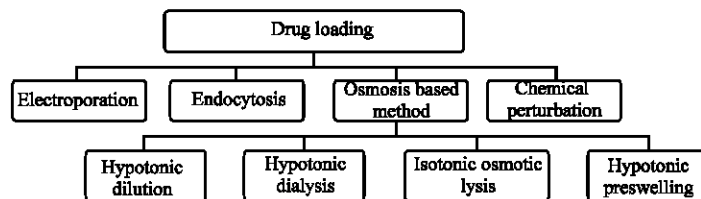


Fig. 1: Schematic illustration for methods of drug loading into erythrocytes

mice (Kravtsoff *et al.*, 1990; Wang *et al.*, 2010), rabbits (Hamidi *et al.*, 2001a), dogs (Tonetti *et al.*, 1991) are used as source for erythrocytes. Freshly collected blood is centrifuged in a refrigerated centrifuge in order to separate packed erythrocytes.

Then packed erythrocytes are washed with isotonic solution several times and centrifugation between washes to remove other blood components. The hematocrit adjusted between 5 and 95%, although the most usual is to work with a hematocrit of 70% (Rossi *et al.*, 2006). The following methods are used for entrapment of the therapeutic agent into erythrocytes (Fig. 1).

**Osmosis-based methods:** Erythrocytes have the ability to undergo reversible swelling and shape changes in a hypotonic solution or under stress. Erythrocytes can increase in volume by 25-50% leading to an initial change in the shape from biconcave to spherical adapt additional volume while keeping the surface area constant (Agnihotri *et al.*, 2010; Gupta *et al.*, 2010). This change is due to the absence of superfluous membrane. Therefore, the cells can maintain their integrity up to a tonicity of  $150 \text{ mosm kg}^{-1}$ , above which the membrane ruptures, releasing the cellular contents. At this point (just before cell lysis), some transient pores of 200-500 Å are generated on the membrane. Erythrocyte ghost is the remnant after cell lysis and depletion of cellular contents which can be resealed by restoring isotonic conditions having the drug inside. Upon incubation, the cells resume their original biconcave shape and recover original impermeability (Briones *et al.*, 2009; Gothoskar, 2004).

**Hypotonic preswelling:** In this technique erythrocytes are incubated in a hypotonic buffered solution to produce swelling and centrifuged at low centrifugation values. The supernatant is discarded and the cell fraction is brought to the lysis point by adding 100-120 µL portions of an aqueous solution of the drug to be encapsulated and centrifugation between the drug addition steps. The tonicity of a cell mixture is restored at the lysis point by adding a calculated amount of hypertonic buffer. Then, the cell suspension is incubated at 37°C to re-anneal the resealed erythrocytes (Gopal *et al.*, 2007).

**Hypotonic dialysis:** The suspension of erythrocytes with hematocrit 50-90% is placed in a dialysis bag facing a hypoosmotic buffer at 4°C. The time of dialysis may vary between 20 and 180 min. Subsequently, an annealing process is performed with the loaded erythrocytes in an isoosmotic medium for 10 min at 37°C. Finally, a resealing of the erythrocytes is performed at 37°C using a hyperosmotic buffer. The hyperosmotic buffer usually contains adenosine, glucose and magnesium chloride (Millan *et al.*, 2004a).

Table 1: Comparison between percent of drug loading, advantages as well as disadvantages of different osmosis based systems (Gopal *et al.*, 2007)

Method	% Loading	Advantages	Disadvantages
Dilution method	20-40	Fastest and simplest especially for low molecular weight drugs	Entrapment efficiency is less
Dialysis	30-45	Better <i>in vivo</i> survival of erythrocytes better structural integrity and membrane	Time consuming, heterogenous size, distribution of resealed erythrocytes
Preswell dilution	30-90	Good retention of cytoplasm and good survival <i>in vivo</i>	-
Isotonic osmotic lysis	-	Better <i>in vivo</i> survival	Impermeable only large molecules, process is time consuming

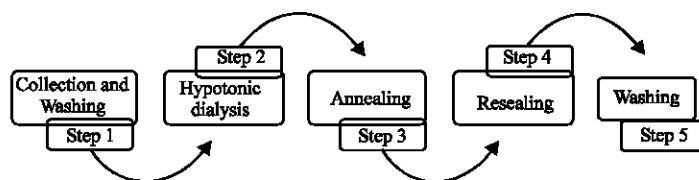


Fig. 2: Scheme represents the details of hypotonic dialysis method

It is based on the principle that semi permeable dialysis membrane maximizes the intracellular/ extracellular volume ratio for macromolecules during lysis and resealing. In this method, the erythrocyte suspension and the drug to be loaded were placed in the blood compartment and the hypotonic buffer was placed in a receptor compartment. This led to the concept of continuous flow dialysis (Gopal *et al.*, 2007).

**Isotonic osmotic lysis:** Isotonic hemolysis can be achieved by physical or chemical means. If erythrocytes are incubated in solutions of a substance with high membrane permeability, the solute will diffuse into the cells because of the concentration gradient. This process is followed by an influx of water to maintain osmotic equilibrium. Chemicals such as urea solution, polyethylene glycol and ammonium chloride have been used for isotonic hemolysis. However, this method also is not immune to changes in membrane structure composition. The suspension was diluted with an isotonic-buffered drug solution. After the cells were separated, they were resealed at 37°C (Jaitely *et al.*, 1996). Figure 2 represents the details of hypotonic dialysis method (Millan *et al.*, 2004b).

**Hypotonic dilution:** Hypotonic dilution was the first method investigated for the encapsulation of chemicals into erythrocytes and is the simplest and fastest. In this method, a volume of packed erythrocytes is diluted with 2-20 volumes of aqueous solution of a drug. The solution tonicity is then restored by adding a hypertonic buffer. The resultant mixture is then centrifuged, the supernatant is discarded and the pellet is washed with isotonic buffer solution (Tajerzadeh and Hamidi, 2000). Comparison between percent of drug loading, advantages as well as disadvantages of different osmosis based systems is shown in Table 1.

**Chemical perturbation of the membrane:** The membrane permeability of erythrocytes is increased when the cells are exposed to certain chemicals like polyene antibiotic such as amphotericin B, halothane also was used for the same purpose (Lin *et al.*, 1999). This induce irreversible destructive changes in the cell membrane (Gupta *et al.*, 2010).

**Electroporation:** This method is based on using transient electrolysis leading to generate pores that produce desirable membrane permeability for drug loading into red blood cells (Gopal *et al.*, 2007). The components can be entrapped when an electric pulse of greater than a threshold voltage of  $1\text{-}10\text{ kV cm}^{-1}$  is applied for  $20\text{-}160\text{ }\mu\text{sec}$  in media and resealed in osmotic medium.

The extent of pore formation depends upon the electric field strength, pulse duration and ionic strength of the suspending medium. Once the membrane is perforated, regardless of the size of the pores, ions rapidly distribute between the extra and intracellular space to attain equilibrium, however the membrane still remain impermeable to its cytoplasmic macromolecules (Hamidi *et al.*, 2007b; Patel, 2009).

**Entrapment by endocytosis:** Endocytosis performed by addition of one volume of washed erythrocytes to nine volumes of buffer containing  $2.5\text{ mM}$  Adenine Triphosphate (ATP),  $2.5\text{ mM}$   $\text{MgCl}_2$  and  $1\text{ mM}$   $\text{CaCl}_2$ , followed by incubation for 2 min at room temperature. The pores created by this method are resealed by using  $154\text{ mM}$  of NaCl and incubation at  $37^\circ\text{C}$  for 2 min. The entrapment of material occurs by endocytosis. The vesicle membrane separates endocytosed material from cytoplasm thus protecting it from the erythrocytes and vice-versa (Harisa *et al.*, 2011). The various candidates entrapped by this method include primaquine (Alanazi, 2010; Alanazi *et al.*, 2011) and related 8-amino-quinolines, vinblastine, chlorpromazine and related phenothiazines, hydrocortisone, propranolol, tetracaine (Alvarez *et al.*, 1998; Hamidi *et al.*, 2007b) and pravastatin (Abdel-Hamid *et al.*, 2011).

## CHARACTERIZATION OF LOADED ERYTHROCYTES

After loading of therapeutic agent on erythrocytes, the carrier cells are exposed to physical, cellular as well as biological evaluations (Table 2) (Patel, 2009).

**Cell counting and cell recovery:** This involves counting the number of red blood cells per unit volume of whole blood, usually by using automated machine. Red cell recovery may be calculated on the basis of the differences in the hematocrit and the volume of the suspension of erythrocytes before and after loading. The goal is to minimize the loss during the encapsulation procedure to maximize cell recovery (Millan *et al.*, 2004a).

**Morphological aspect:** The morphological characterization of erythrocytes is undertaken by comparison with untreated erythrocytes using either transmission (TEM) or Scanning Electron Microscopy (SEM) (Pierige *et al.*, 2008). These techniques are done to detect morphological changes in the erythrocytes induced by encapsulation methods. Thus, when erythrocytes are subjected to isotonic solutions ( $300\text{ mosm kg}^{-1}$ ) they reveal the typical morphology of discocyte (biconcave). This evolves to a morphology of stomatocyte (uniconcave) when they are subjected to solutions of  $200\text{ mos M kg}^{-1}$ , attaining the spherocytic shape (the most fragile of the three) when the solution is of  $150\text{ mosm kg}^{-1}$  (Hamidi *et al.*, 2007a; Magnani and DeLoach, 1992).

**Osmotic behavior:** This test is done to detect the effect of loading process on the fragility of red blood cells to check the status of erythrocytes' membrane. Unloaded and loaded erythrocytes are tested by exposure to different concentration of sodium chloride, making them swell, in order to determine the relative fragility of the red cells Turbulence shock (Bektas and Ayik, 2009; Abdelhalim and Moussa, 2010).



Table 2: Summary of characterization parameters and their determination for carrier erythrocytes

Parameter	Method/instrument used
<b>Physical characterization</b>	
Shape and surface morphology	Transmission electron microscopy, Scanning electron microscopy, Phase contrast microscopy, Optical microscopy
Vesicle size and size distribution	Transmission electron microscopy, Optical microscopy
Drug release	Diffusion cell, dialysis
Drug content	Deproteinization of cell membrane followed by assay of resealed drug, radiolabelling
Surface electrical potential	Zeta potential measurement
Surface pH	pH sensitive probes
Deformability	Capillary method
<b>Cellular characterization</b>	
% Hb content	Deproteinization of cell membrane followed by hemoglobin assay
Cell volume	Laser light scattering
% Cell recovery	Neubauer's chamber, hematological analyzer
Osmotic fragility	Stepwise incubation with isotonic to hypotonic saline solutions and determination of drug and hemoglobin assay
Osmotic shock	Dilution with distilled water and estimation of drug and hemoglobin
Turbulent shock	Passage of cell suspension through 30-gauge hypodermic needle at 10 ml/min flow rate and estimation of residual drug and hemoglobin. Vigorous shaking followed by hemoglobin estimation
Erythromycin sedimentation rate	ESR method
<b>Biological characterization</b>	
Sterility	Sterility test
Pyrogenicity	Rabbit method
Animal toxicity	Toxicity tests

This test is done to evaluate the stability of the loaded erythrocytes against the turbulence stress exerted by the cells against *in vivo* circulation turbulence (Millan *et al.*, 2004a). Packed erythrocytes are suspended in 10 mL of PBS in polypropylene test tubes and are shaken vigorously using a multiple test tubes orbital shaker at 2000 rpm for 4 h. To determine the time course of hemoglobin release, 0.5 mL portions of each suspension were withdrawn at 0, 0.5, 1, 2 and 4 h elapsed and after centrifuging at 1000×g for 10 min. The absorbances of the supernatants are determined spectrophotometrically at 540 nm. The percent of hemoglobin release is determined in reference to a completely lysed cell suspension with the same cell fraction (i.e., 0.5 mL packed cells added to 10 mL of distilled water). To compare the turbulence fragilities of the different types of erythrocytes, a turbulence fragility index is defined as the shaking time producing 20% hemoglobin release from erythrocytes (Hamidi *et al.*, 2007a):

$$\text{Percent hemolysis (\%)} = \frac{\text{Absorbance of the test supernatant}}{\text{Absorbance of control}} \times 100 \text{ (Chikezie, 2011)}$$

***In vitro* drug release:** The drug loading may produce sustained release of the drug that influences the pharmacokinetic behavior *in vivo* of the loaded erythrocytes. *In vitro* leakage of the drug from loaded erythrocytes is tested using autologous plasma or an isoosmotic buffer at 37°C with a hematocrit adjusted between 0.5 and 50%. The supernatant is removed at the time intervals previously programmed and replaced by an equal volume of autologous plasma or buffer (Magnani and DeLoach, 1992).

Some authors recommend performing in vitro the release studies from loaded erythrocytes using a dialysis bag (Millan *et al.*, 2004a). The drug release is controlled by molecular weight and liposolubility of the drug (Hamidi *et al.*, 2007b). Lipophilic drugs may be released from the red cells by a mechanism of passive diffusion, while hydrophilic drugs need cell lysis to be released (Hamidi *et al.*, 2001b).

**Hemoglobin release:** The content of hemoglobin of the erythrocytes may be diminished by the alterations in the permeability of the membrane of the red cells during the encapsulation procedure (Hamidi *et al.*, 2001b). Furthermore, the relationship between the rate of hemoglobin and the rate of drug release contributes to interpreting the mechanisms involved in the release of the substance encapsulated from the erythrocytes (Hamidi *et al.*, 2001b; Pierige *et al.*, 2008). The hemoglobin leakage is tested using a red cell suspension by recording the absorbance of supernatant at 540 nm on a spectrophotometer (Millan *et al.*, 2004a).

**Biological characterization:** Biological characterization of the developed erythrocytes includes sterility test, pyrogenicity test and toxicity tests (Gopal *et al.*, 2007).

#### **IN VITRO STORAGE OF CARRIER ERYTHROCYTES**

Preparing drug-loaded erythrocytes on a large scale and maintaining their survival and drug content can be achieved by using suitable storage methods. The most common storage media include Hank's balanced salt solution and acid-citrate-dextrose at 4°C. Cells remain viable in terms of their physiologic and carrier characteristics for at least 2 weeks at this temperature. The addition of calcium-chelating agents or the purine nucleosides improve circulation survival time of cells upon reinjection (Gopal *et al.*, 2007; Millan *et al.*, 2004a).

#### **SAFETY CONSIDERATION IN CARRIER ERYTHROCYTES**

The safety of utilization of erythrocytes as carrier has been illustrated in our previous report (Adams *et al.*, 2003) and summary of this consideration shows in Table 3. The use of erythrocytes as a drug carrier in human has the inherited problems of transfusion of blood from one to another. If two different blood types are mixed together, the blood cells may begin to clump together in the blood vessels, causing a potentially fatal situation. Therefore, it is important to identify the blood type of the acceptor and the type of erythrocyte carrier to minimize mismatching before the administration of drug-loaded erythrocytes takes place. Another inherited problem is the risk of transmitting diseases. Therefore, screening of these carriers for the absence of diseases is important to eliminate any risk of contamination.

Utilization of erythrocyte as a drug carrier raises another potential concern due to the changes in their biochemical nature. In some instances such changes created therapeutic benefits whereas in other cases they yielded unwanted results. For example, Hamidi *et al.* (2001b) conducted a study on erythrocytes loaded with enalaprilat (Hamidi *et al.*, 2001b). The process produced erythrocytes that were more rigid, less deformed and more therapeutically efficacious than unloaded erythrocytes. The modification of erythrocytes with proteins such as streptavidin, however, elicited some negative results. The attachment of streptavidin to biotinylated red blood cells caused these cells to be lysed, rapidly cleared from the circulation thereby reducing their biocompatibility (Muzykantov *et al.*, 1996). *In vivo* studies involving humans and animals have also been conducted

Table 3: Safety consideration in carrier erythrocytes

Step to be considered	Safety issue
Different blood types	Blood clotting and clotting
Possible risk of contamination	HIV, HBV, etc
Changing on physical and possible of clotting	Rigidity of the membrane modification on erythrocytes membrane's biochemical characteristics proteins lead to lysis Extensive biotinylated leads to rapid elimination and kidney problems
Changing in pharmacokinetics and dynamic behaviors of the loaded drug	Increase the production of unfavorable metabolites

on biotinylated red blood cells. Extensive biotinylation severely altered the biocompatibility of these cells causing rapid elimination whereas moderate biotinylation generated stable erythrocytes that circulated for several hours (Muzykantov *et al.*, 1996).

Encapsulation of drug in erythrocytes alters their pharmacokinetics properties and changes their metabolic pathway. Thus, in some cases using erythrocytes as drug carriers resulted in undesirable cytotoxicity. Doxorubicin encapsulated in erythrocytes treated with glutaraldehyde was more toxic than the parent compound (Kohane *et al.*, 2002). The drug-encapsulated erythrocytes may also increase the production of unfavorable metabolites. For example, doxorubicinol, a toxic metabolite of doxorubicin, was produced in higher quantity when doxorubicin-encapsulated erythrocytes were administrated.

## CONCLUSION

Carrier erythrocytes are one of biological drug delivery systems have been investigated in recent decades that covered a wide variety of drugs and other bioactive agents. This is generally due to their notable degree of biodegradability, biocompatibility, availability and ease of preparation and use. The controlled and/or targeted release of active agents is among the mostly attractive applications of erythrocyte carriers in drug delivery. In this review, different methods of loading, characterization, applications as well as usage safety have been summarized.

## ACKNOWLEDGMENT

The author gratefully acknowledge the generous financial support from the Deanship of Scientific Research grant No. NPAR3-(2).

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