Available online at www.ijpsdr.com
International Journal of Pharmaceutical Sciences and Drug Research 2010; 2(1): 23-30

Review Article ISSN 0975-248X

Cell Based Drug Delivery System through Resealed Erythrocyte - A Review

A Gupta[1*], AK Mishra[1], P Bansal[1], S Kumar[1], V Gupta[1], R Singh[2], GS Kalyan[3]

[1]*National Institute of Ayurvedic Pharmaceutical Research, CCRAS, Patiala, Punjab, India*
[2]*Shobhit University, School of Pharmaceutical Sciences, Meerut, Uttar Pradesh, India*
[3]*Government Medical College, Patiala, Punjab, India*

ABSTRACT
Erythrocytes are biocompatible, biodegradable, possess long circulation half lives, and can be loaded with a variety of biologically active compounds using various chemical and physical methods. Erythrocytes are potential biocompatible vectors for different bioactive substances, including drugs. These can be used successfully as biological carriers of drugs, enzymes and peptides. There are currently diverse methods that permit drug encapsulation in erythrocytes with an appropriate yield. Erythrocytes loaded with drugs and other substances allow for different release rates to be obtained. Encapsulation in erythrocytes significantly changes the pharmacokinetic properties of drugs in both animals and humans, enhancing liver and spleen uptake and targeting the reticulo-endothelial system (RES). This review explains the different method of drug loading and their characterization parameters for resealed erythrocytes. This method of drug targeting is safe and effective for longer period of time and beneficial for the society.

Keywords: Carrier erythrocytes, Drug targeting, Cellular carriers, Resealed erythrocytes.

INTRODUCTION

At present, there are 30 main drug delivery products on the market. The total annual income for all of these is approximately US$33 billion with an annual growth of 15 % (based on global product revenue). The reasons for this increasing interest in drug delivery are due to the increasing need of safe drugs, capable of reaching the target and with minimal side effects. In fact the main problems associated with systemic drug administration are essentially related to the bio-distribution of pharmaceuticals throughout the body. This indiscriminate distribution means that, to achieve a required therapeutic concentration the drug has to be administered in large quantities, the major part of which is just wasted in normal tissues. Ideally, a "perfect" drug should exert its pharmacological activity only at the target site, using the lowest concentration possible and without negative effects on non-target compartments. The delivery systems currently available enlist carriers that are either simple, soluble macromolecules (such as monoclonal antibodies, soluble synthetic polymers, polysaccharides and particulate biodegradable polymers) or more complex multicomponent structures (microcapsules, Microparticles, cells, cell ghosts, lipoproteins, liposomes, erythrocytes). Drug delivery either by means of soluble or particulate components can affect drug pharmacokinetic, drug bioavailability, sometime safety and efficacy however only rarely reach the main goal of having the drug targeted only to the site were its action should be exerted. Thus, an additional advantage of modern delivery system is sometime associated with the possibility of having selected targeting properties useful to increase drug selectivity and improve drug efficacy. Unfortunately, sometime the body recognizes the drug targeting system as non-self and unexpected toxicities could hamper the use of the same. This is the case of the first generation of monoclonal antibodies coupled to cytotoxic drugs or of other soluble carriers experimented at preclinical level. It has been envisaged that ideal drug delivery systems should be made of self-powered, computer-controlled medical nanorobot system, named pharmacyte [1], capable of precise transport, timing, and targeted delivery of pharmaceutical agents to specific target in the body. This ideal drug delivery system is not yet available but significant progress has been made in the last years over the traditional drug formulations and, in our opinion, the cell based delivery systems are the closest ones to the ideal drug delivery system named above.

***Corresponding author: Mr. A Gupta,**
National Institute of Ayurvedic Pharmaceutical Research, CCRAS, Patiala, Punjab, India; **Tel:** 09023468460, 0902346763; **E-mail:** abhibbd2006@gmail.com, vikas_4308@rediffmail.com

Among the cell based delivery systems two categories could be identified:

1. **Transduced cells**, capable of expressing pharmaceutically relevant agents.
2. **Cell carriers** which could be loaded with drugs or therapeutics. In this category the carrier cells could release he drug content in circulation or at selected sites or could target the drug to other relevant cells in the body.

CELL CARRIERS

Erythrocytes, also known as red blood cells, have been extensively studied for their potential carrier capabilities for the delivery of drugs and drug-loaded microspheres. [2-4] Such drug-loaded carrier erythrocytes are prepared, simply by collecting blood samples from the organism of interest, separating erythrocytes from plasma, entrapping drug in the erythrocytes, and resealing the resultant cellular carriers. [2] Hence, these carriers are called resealed erythrocytes. The overall process is based on the response of these cells under osmotic conditions. Upon reinjection, the drug-loaded erythrocytes serve as slow circulating depots and target the drugs to a reticulo-endothelial system (RES). [3-6]

MORPHOLOGY AND PHYSIOLOGY OF ERYTHROCYTES

Erythrocytes are the most abundant cells in the human body (5.4 million cells/mm^3 blood in a healthy male and 4.8 million cells/mm^3 blood in a healthy female). These cells were described in human blood samples by Dutch Scientist Lee Van Hock in 1674. In the 19^{th} century, Hope Seyler identified hemoglobin and its crucial role in oxygen delivery to various parts of the body. [7] Erythrocytes are biconcave discs with an average diameter of 7.8 µm, a thickness of 2.5 µm in periphery, 1 µm in the center, and a volume of 85-91 μm^3. [8] The flexible, biconcave shape enables erythrocytes to squeeze through narrow capillaries, which may be only 3 µm wide. Mature erythrocytes are quite simple in structure. They lack a nucleus and other organelles. Their plasma membrane encloses hemoglobin, a heme-containing protein that is responsible for O_2–CO_2 binding inside the erythrocytes. The main role of erythrocytes is the transport of O_2 from the lungs to tissues and the CO_2 produced in tissues back to lungs. Thus, erythrocytes are a highly specialized O_2 carrier system in the body. Because a nucleus is absent, all the intracellular space is available for O_2 transport. Also, because mitochondria are absent and because energy is generated anaerobically in erythrocytes, these cells do not consume any of the oxygen they are carrying. Erythrocytes live only about 120 days because of wear and tear on their plasma membranes as they squeeze through the narrow blood capillaries. Worn-out erythrocytes are removed from circulation and destroyed in the spleen and liver (RES), and the breakdown products are recycled. The process of erythrocyte formation within the body is known as *erythropoiesis*. In a mature human being, erythrocytes are produced in red bone marrow under the regulation of a hemopoietic hormone called erythropoietin. [9]

RED BLOOD CELLS

Human RBCs are the most common cells of blood, are responsible for oxygen transport and have a typical biconcave shape. Normal human RBCs have a diameter of 7–8 µm and an average volume of 90 fl. In mammals, RBCs are anucleated and lose their organelles during maturation. Oxygen transport is guaranteed by about 270 million haemoglobin molecules per cell each of which with four heme groups. A human body is commonly endowed with 2-3 10^{13} RBCs continuously produced at a rate of 2 million per second. In fact, RBCs spent its 100-120 day life-span travelling the circulatory system before being selectively removed by macrophages in the reticulo-endothelial system (RES). [10] The surface area of mature, biconcave RBCs is about 136 μm^2 but can swell to a sphere of approx 150 fl. It is noteworthy that RBCs can also cross undamaged capillaries of 2-3 µm in diameter. The RBC membrane is strictly connected with the membrane skeletal proteins which are organized in a uniform shell. The RBC shape can undergo a number of reversible transformations. An important determinant of RBC survival is its deformability. Key factors affecting deformability are internal viscosity (mainly contributed by RBC haemoglobin), the surface/volume of the cell and the intrinsic deformability of the membrane. The RBCs have other very interesting properties namely they behave as an osmometer since they shrink when placed into a hypertonic solution or swell when placed into a hypotonic solution. The RBCs can reach a critical haemolytic volume giving rise to holes on the membrane ranging from 10 nm up to 500 nm. These processes are usually reversible and following haemolysis the holes close and the cell resumes its biconcave shape. Red blood cells constitute potential biocompatible carriers for different bioactive substances, including protein drugs, since they feature some unique advantages [11-13], for examples, they are completely biodegradable without generation of toxic.

SOURCE OF ERYTHROCYTES

Various types of mammalian erythrocytes have been used for drug delivery, including erythrocytes of mice, cattle, pigs, dogs, sheep, goats, monkeys, chicken, rats, and rabbits.

ISOLATION OF ERYTHROCYTES

Erythrocytes may be prepared as carriers from blood taken from human beings [14] and from different animal species, such as rats [15], mice [16], rabbits [17], dogs [18], etc. Blood is taken from the human being, mouse, rat, or the animal species in question, using a suitable anti-coagulant. Application is normally made of EDTA, as it is the anticoagulant that best preserves the properties of blood cells. [19] Freshly collected blood is centrifuged in a refrigerated centrifuge in order to separate packed erythrocytes. Several washes are subsequently performed. This is a process that normally involves repeated centrifugation with an isoosmotic solution to remove other blood components. [20] The erythrocytes can be washed more efficiently by using a capillary hollow fibre plasma separator. [21] The hematocrits employed may be variables ranging between 5 % and 95 % [22-23], although the most usual is to work with a haematocrit of 70 %. [24] In 1953, Gardos tried to load erythrocyte ghost using adenosine triphosphate (ATP). [25] In 1959, Marsden and Ostting reported the entrapment of dextran (molecular weight 10–250 kDa). In 1973, the loading of drugs in erythrocytes was reported separately by Ihler et al. [26] and Zimmermann. [27] In 1979, the term carrier erythrocytes were coined to describe drug-loaded erythrocytes. [6]

ADVANTAGES OF ERYTHROCYTES IN DRUG DELIVERY

A remarkable degree of biocompatibility, particularly when the autologous cells are used for drug loading [28-36], Complete biodegradability and the lack of toxic product(s) resulting from the carrier biodegradation [28, 30, 36-37],

Avoidance of any undesired immune responses against the encapsulated drug [38], Considerable protection of the organism against the toxic effects of the encapsulated drug, e.g., antineoplasms [39], Remarkably longer life-span of the carrier erythrocytes in circulation in comparison to the synthetic carriers [29, 30, 35]. In the optimum condition of the loading procedure, the life-span of the resulting carrier cells may be comparable to that of the normal erythrocytes [32, 40-41], An easily controllable life-span within a wide range from minutes to months [33], Desirable size range and the considerably uniform size and shape [32,42-43], Protection of the loaded compound from inactivation by the endogenous factors; [30, 35, 37, 39, 44], Possibility of targeted drug delivery to the RES organs [28, 30, 41], Relatively inert intracellular environment [38], Availability of knowledge, techniques, and facilities for handling, transfusion, and working with erythrocytes [28, 30], Possibility of an ideal zero-order kinetics of drug release [44], Wide variety of compounds with the capability of being entrapped within the erythrocytes; [30, 39, 45-47], Possibility of loading a relatively high amount of drug in small volume of erythrocytes, which, in turn, assures the dose sufficiency in clinical as well as animal studies using a limited volume of erythrocyte samples [30, 36], Modification of the pharmacokinetic and pharmacodynamic parameters of the drug [31, 34, 37, 39], Remarkable decrease in concentration fluctuations in steady state in comparison to the conventional methods of drug administration [28, 31, 34, 48-49], which is a common advantage for most of the novel drug delivery systems, Considerable increase in drug dosing intervals with drug concentration in the safe and effective level for a relatively long time [28, 34, 41, 48], Possibility of decreasing drug side effects. [28, 34, 49]

DISADVANTAGES OF ERYTHROCYTES IN DRUG DELIVERY

The major problem encountered in the use of biodegradable materials or natural cells as drug carriers is that they are removed *in vivo* by the RES. This seriously limits their useful life as drug carriers and in some cases may pose toxicological problems, The rapid leakage of certain encapsulated substances from the loaded erythrocytes, Several molecules may alter the physiology of the erythrocyte, Given that they are carriers of biological origin, encapsulated erythrocytes may present greater variability and lesser standardisation in their preparation, compared to other carrier systems, The storage of the loaded erythrocytes is a further problem involving carrier erythrocytes for their possible use in therapeutics. Tests have been performed on their conditioning in suspension in isotonic buffers containing all essential nutrients, as well as in low temperatures, with the addition of nucleosides or chelators, liophylisation, freezing with glycerol or gel immobilization, Liable to biological contamination due to the origin of the blood, the equipment and the environment, such as air. Rigorous controls are required accordingly for the collection and handling of the erythrocytes. [50-55]

LOADING METHODS

Methods of drug loading

Several methods have been reported for encapsulation of drug or other bioactive agents in erythrocytes. Some of these methods such as electrical pulse methods and osmosis-based methods have a physical nature whereas the other methods such as the chemical perturbation of the membrane are chemically based. Regardless of the method used, the optimal characteristics for a compound to be encapsulated successfully in erythrocytes include a considerable degree of water solubility, resistance against inactivation within the erythrocytes, the lack of physical and/or chemical interaction with erythrocyte membrane or the other cell constituents, and well-defined pharmacokinetic as well as pharmacodynamic properties [29, 56]. Hypotonic hemolysis [31, 46, 57], hypotonic dilution [28, 30, 32, 37-38, 45-46, 58-63], hypotonic dialysis [29-30, 44, 46, 56, 62, 64-69], hypotonic preswelling [30-31, 33, 36, 40, 43, 46, 49, 59-60, 70-73], and osmotic pulse [74] methods are categorized as osmosis-based methods. Chemical perturbation of the membrane [29, 75], electrical breakdown or 'electroporation' [28, 29, 30, 41, 48, 76-78], endocytosis [46], lipid fusion [29, 30], laser loading [78], and intrinsic uptake of substances by erythrocytes [79] are other reported methods used for encapsulation of drugs and other agents into erythrocytes.

Hypotonic hemolysis

This method is based on the ability of erythrocytes to undergo reversible swelling in a hypotonic solution. Erythrocytes have an exceptional capability for reversible shape changes with or without accompanying volume change and for reversible deformation under stress. An increase in volume leads to an initial change in the shape from biconcave to spherical. This change is attributable to the absence of superfluous membrane; hence the surface area of the cell is fixed. The cells assume a spherical shape to accommodate additional volume while keeping the surface area constant. The volume gain is 25–50 %. The cells can maintain their integrity up to a tonicity of ~150 mosm/kg, 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. After cell lysis, cellular contents are depleted. The remnant is called an erythrocyte ghost. [6, 80-82] The principle of using these ruptured erythrocytes as drug carriers is based on the fact that the ruptured membranes can be resealed by restoring isotonic conditions. Upon incubation; the cells resume their original biconcave shape and recover original impermeability. [80-82]

Use of red cell loader

A novel method for entrapment of non-diffusible drugs into erythrocytes by red cell loader method. [83] With as little as 50 mL of a blood sample, different biologically active compounds were entrapped into erythrocytes within a period of 2 h at room temperature under blood banking conditions. The process is based on two sequential hypotonic dilutions of washed erythrocytes followed by concentration with a hemofilter and an isotonic resealing of the cells. There was ~30 % drug loading with 35-50 % cell recovery. The processed erythrocytes had normal survival in vivo. The same cells could be used for targeting by improving their recognition by tissue macrophages. [83]

Hypotonic dilution

Hypotonic dilution was the first method investigated for the encapsulation of chemicals into erythrocytes [26] and is the simplest and fastest. [6] 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. [26, 80] The major drawbacks of this method include low entrapment efficiency [26, 84-87] and a considerable loss of hemoglobin and other cell components. [5, 88-89] This reduces the circulation half life of

the loaded cells. These cells are readily phagocytosed by RES macrophages and hence can be used for targeting RES organs. [26, 80] Hypotonic dilution is used for loading enzymes such as β-galactosidase and β-glucosidase [26], asparginase [90-91], and arginase [92], as well as bronchodilators such as salbutamol. [93]

Hypotonic pre-swelling

This method was developed by Rechsteiner [94] in 1975 and was modified by Jenner et al. for drug loading. The technique is based upon initial controlled swelling in a hypotonic buffered solution. This mixture is centrifuged at low *g* 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. The mixture is centrifuged between the drug-addition steps. The lysis point is detected by the disappearance of a distinct boundary between the cell fraction and the supernatant upon centrifugation. 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 reanneal the resealed erythrocytes. [89, 94] Such cells have a circulation half life comparable to that of normal cells. [6, 80, 85, 95] This method is simpler and faster than other methods, causing minimum damage to cells. Drugs encapsulated in erythrocytes using this method include propranolol [96], asparginase [97], cyclopohphamide, cortisol-21-phosphate [85, 98], ώ1-antitrypsin [85], metronidazole [87], levothyroxine [95], methotrexate, insulin [85, 99], enalaprilat [100], and isoniazid. [101]

Hypotonic dialysis

This method was first reported by Klibansky [102] in 1959 and was used in 1977 by Deloach and Ihler [86], and Dale [103] for loading enzymes and lipids. Several methods are based on the principle that semi permeable dialysis membrane maximizes the intracellular: extracellular volume ratio for macromolecules during lysis and resealing. In the process, an isotonic, buffered suspension of erythrocytes with a hematocrit value of 70–80 is prepared and placed in a conventional dialysis tube immersed in 10–20 volumes of a hypotonic buffer. The medium is agitated slowly for 2 h. The tonicity of the dialysis tube is restored by directly adding a calculated amount of a hypertonic buffer to the surrounding medium or by replacing the surrounding medium by isotonic buffer. [80, 86] The drug to be loaded can be added by either dissolving the drug in isotonic cell suspending buffer inside a dialysis bag at the beginning of the experiment [80, 104-105] or by adding the drug to a dialysis bag after the stirring is complete. [81, 86, 106-109] The use of standard hemodialysis equipment for loading a drug in erythrocytes was reported by Roper et al. [110] 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," which has been used by several other researchers. [81, 105, 110-115] The loaded cells exhibit the same circulation half life as that of normal cells. [5-6] Also, this method has high entrapment efficiency on the order of 30-50 % [5, 81, 84, 86], cell recovery of 70-80 %, high-loading capacity [50, 54], and is amenable to automation with control of process variables. [33, 79] The drawbacks include a long processing time [5, 84, 86] and the need for special equipment. [80] This method has been used for loading enzymes such as β-galactosidase, glucoserebrosidase [85], asparginase [105], inositol hexaphosphatase [112, 114-115], as well as drugs such as gentamicin [89], adriamycin [104], pentamidine and furamycin [107], interlukin-2 [108], desferroxamine [106, 108-111, 113], and human recombinant erythropoietin. [116]

Isotonic osmotic lysis

This method, also known as the osmotic pulse method, involves isotonic hemolysis that is achieved by physical or chemical means. The isotonic solutions may or may not be isoionic. 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 [117], polyethylene glycol [118], and ammonium chloride have been used for isotonic hemolysis. However, this method also is not immune to changes in membrane structure composition. In 1987, Franco et al. developed a method that involved suspending erythrocytes in an isotonic solution of dimethyl sulfoxide (DMSO). [119] The suspension was diluted with an isotonic-buffered drug solution. After the cells were separated, they were resealed at 37°C.

Chemical perturbation of the membrane

This method is based on the increase in membrane permeability of erythrocytes when the cells are exposed to certain chemicals. In 1973, Deuticke et al. showed that the permeability of erythrocytic membrane increases upon exposure to polyene antibiotic such as amphotericin B. [120] In 1980, this method was used successfully by Kitao and Hattori to entrap the antineoplastic drug daunomycin in human and mouse erythrocytes. [121] Lin et al. [122] used halothane for the same purpose. However, these methods induce irreversible destructive changes in the cell membrane and hence are not very popular.

Electro-insertion or electroencapsulation

In 1973, Zimmermann tried an electrical pulse method to encapsulate bioactive molecules. [27] Also known as electroporation, the method is based on the observation that electrical shock brings about irreversible changes in an erythrocyte membrane. In 1977, Tsong and Kinosita suggested the use of transient electrolysis to generate desirable membrane permeability for drug loading. [123] The erythrocyte membrane is opened by a dielectric breakdown. Subsequently, the pores can be resealed by incubation at 37°C in an isotonic medium. The procedure involves suspending erythrocytes in an isotonic buffer in an electrical discharge chamber. A capacitor in and the sophistication of the process. [4, 81, 84] Entrapment efficiency of this method is ~35 % [30], and the life span of the resealed cells in circulation is comparable with that of normal cells. [5] Various compounds such as sucrose [124], urease [124], methotrexate [125], isoniazid [126], human glycophorin [127], DNA fragments, and latex particles of diameter 0.2 µm [5] can be entrapped within erythrocytes by this method. Mangal and Kaur achieved sustained release of a drug entrapped in erythrocytes with the use of electroporation. [128]

Entrapment by endocytosis

This method was reported by Schrier et al. in 1975. [129] Endocytosis involves the addition of one volume of washed packed erythrocytes to nine volumes of buffer containing 2.5 mM ATP, 2.5 mM $MgCl_2$, and 1mM $CaCl_2$, followed by incubation for 2 min at room temperature. The pores created by this method are resealed by using 154 mM of NaCl and incubation at 37°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. The various candidates entrapped by this method include primaquine and related 8–amino–quinolines, vinblastine, chlorpromazine and related phenothiazines, hydrocortisone, propranolol, tetracaine, and vitamin A. [123, 130-131]

Loading by electric cell fusion

This method involves the initial loading of drug molecules into erythrocyte ghosts followed by adhesion of these cells to target cells. The fusion is accentuated by the application of an electric pulse, which causes the release of an entrapped molecule. An example of this method is loading a cell-specific monoclonal antibody into an erythrocyte ghost. [125, 132] An antibody against a specific surface protein of target cells can be chemically cross-linked to drug-loaded cells that would direct these cells to desired cells.

Loading by lipid fusion

Lipid vesicles containing a drug can be directly fused to human erythrocytes, which lead to an exchange with a lipid-entrapped drug. This technique was used for entrapping inositol monophosphate to improve the oxygen carrying capacity of cells. [133] However, the entrapment efficiency of this method is very low (~1 %).

EVALUATION

In-vitro properties of loaded erythrocytes

1. Cell counting and cell recovery

This involves counting the number of red blood cells per unit volume of whole blood, usually by automated counting. 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. [134]

2. Morphological aspect

The morphological examination of these ghost erythrocytes is undertaken by comparison with untreated erythrocytes using either transmission (TEM) or scanning (SEM) electron microscopy. By means of electron microscopy observation may be made of the morphological changes in the erythrocytes induced by osmosis-based encapsulation methods, when they are subjected to solutions of different osmolality. Thus, when rat erythrocytes are subjected to isotonic solutions (300 mosM/kg) they reveal the typical morphology of discocyte (biconcave). This evolves to a morphology of stomatocyte (uniconcave) when they are subjected to solutions of 200 mosM/kg, attaining the spherocytic shape (the most fragile of the three) when the solution is of 150 mosM/kg. [134]

3. Osmotic fragility

Osmotic fragility is a test to detect abnormal fragility of red blood cells. Untreated or loaded erythrocytes are tested by exposure to hypotonic solutions, making them swell, in order to determine the relative fragility of the red cells [135-136].

4. Turbulence shock

Turbulence shock enables an evaluation to be made of the stability of the loaded erythrocytes against the turbulence stress exerted by the cells against in-vivo circulation turbulence. [135] The test is performed by the method of Deloach et al. [137] whereby the suspension of cells is passed several times through a 22-gauge needle.

5. In-vitro drug or peptide release

The encapsulation of many drugs in erythrocytes can give rise to a sustained release of the drug that influences the pharmacokinetic behaviour 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. [138-139] Certain authors recommend performing in vitro the release studies from loaded erythrocytes using a dialysis bag. [140] The molecular weight and liposolubility of the substance constitute two factors that have a decisive bearing on the release profile of the active principle from the loaded erythrocytes. [141] Liposoluble drugs may be released from the red cells by a mechanism of passive diffusion. Other drugs may become attached to cell structures and are not released by the diffusion mechanism, requiring the lysis of the cell. [142] Band 3 and glycophorin A are proteins present in high density on the extra-cellular surface of erythrocytes and which may act as potential targets for anchoring via covalent bond formation with different substances. Band 3 plays an important role as a carrier protein for anions. [143]

6. Haemoglobin 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. [144-146] 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. [142] The hemoglobin leakage is tested using a red cell suspension by recording the absorbance of supernatant at 540 nm on a spectrophotometer. [135]

7. In vitro stability

The stability of the loaded erythrocytes is assessed by means of the incubation of the cells in autologous plasma or in an isoosmotic buffer, setting the hematocrit between 0.5% and 5% at temperatures of 4 and 37^{o}C. [147- 149]

The mechanism of resealed erythrocytes shows potential for a safer and sure delivery of various drugs for active and passive targeting. The authors have reviewed the explanations of the different method of drug loading and their characterization parameters for resealed erythrocytes. However more research and inputs are required for the cell based drug delivery systems to open a new perspective to the possibility of using cells for therapeutic purposes. The authors are confident that erythrocytes have great potentialities in the field of drug delivery, since the key to success of much therapeutics greatly depends on the development of novel technologies to improve and control the delivery of drugs.

REFERENCES

1. Freitas RA. Pharmacytes: an ideal vehicle for targeted drug delivery. J. Nanosci. Nanotechnol. 2006; 6: 2769–2775.
2. Green R and Widder KJ. Methods in Enzymology. Academic Press, San Diego, 1987, pp. 149.
3. Ropars C, Chassaigne M, Nicoulau C. Advances in the BioSciences. Pergamon Press, Oxford, 1987, pp. 67.
4. Lewis DA, Alpar HO. Therapeutic Possibilities of Drugs Encapsulated in Erythrocytes. Int. J. Pharm. 1984; 22: 137–146.
5. Zimmermann U. Cellular Drug-Carrier Systems and Their Possible Targeting In Targeted Drugs. John Wiley & Sons, New York, 1983, pp. 153–200.

6. Jain S, Jain NK. Engineered Erythrocytes as a Drug Delivery System. Indian J. Pharm. Sci. 1997; 59: 275–281.
7. Telen MJ, Lee R. "The Mature Erythrocytes," in Winthrob's Clinical Hematology. Edn. 9th, Lea & Febiger, Philadelphia, PA, 1993, pp. 101–133.
8. Guyton AC, Hall JE. "Red Blood Cells,Anemia and Polycytemia," in Textbook of Medical Physiology. W.B. Saunders, Philadelphia, PA, 1996, pp. 425–433.
9. Torotra GJ, Grabowski SR. "The Cardiovascular System: The Blood," in Principles of Anatomy and Physiology. Edn. 7th, Harper Collins College Publishers, New York, NY, 1993, pp. 566–590.
10. Beutler E, Lichtman MA. Williams Hematology, Edn. Fifth, McGraw-Hill Inc., New York, 1995, pp. 349–425.
11. Rossi L, Serafini S, Pierigé F, Antonelli A, Cerasi A, Fraternale A, Chiarantini L, Magnani M. Erythrocyte-based drug delivery. Expert Opin. Drug Deliv. 2005; 2: 311–322.
12. Serafini S, Rossi L, Antonelli A, Fraternale A, Cerasi A, Crinelli R, Chiarantini L, Schiavano GF, Magnani M. Drug delivery through phagocytosis of red blood cells. Transfus. Med. Hemother. 2004; 31: 92–101.
13. Gutiérrez Millán C, Salayero Marinero ML, Zarzuelo Castañeda A, Lanao JM. Drug, enzyme and peptide delivery using erythrocytes as carriers. J. Control. Release. 2004; 95: 27–49.
14. Magnani M, Rossi L, Dascenzo M, Panzani I, Bigi L, Zanella A. Erythrocyte engineering for drug delivery and targeting. Biotechnol. Appl. Biochem. 1998; 28: 1– 6.
15. Mishra PR, Jain NK. Biotinylated methotrexate loaded erythrocytes for enhanced liver uptake ''A study on the rat''. Int. J. Pharm. 2002; 231 (2): 145– 153.
16. Kravtzoff R, Ropars C, Laguerre M, Muh JP, Chassaigne M. Erythrocytes as carriers for L-asparaginase. Methodological and mouse in-vivo studies. J. Pharm. Pharmacol. 1990; 42 (7): 473–476.
17. Hamidi M, Tajerzadeh H, Dehpour AR. Inhibition of serum angiotensin-converting enzyme in rabbits after intravenous administration of enalaprilatloaded intact erythrocytes. J. Pharm. Pharmacol. 2001; 53 (9): 1281– 1286.
18. Tonetti M, Astroff AB, Satterfield W, De Flora A, Benatti U, DeLoach JR. Pharmacokinetic properties of doxorubicin encapsulated in glutaraldehyde-treated canine erythrocytes. Am. J. Vet. Res. 1991; 52: 1630– 1635.
19. Green R, Widder KJ. Methods in Enzymology. vol. 149, Academic Press, San Diego, 1987, pp. 221– 229.
20. DeLoach JR, Ihler G. A dyalisis procedure for loading erythrocytes with enzymes and lipids. Biochim. Biophys. Acta. 1977; 496: 136–145.
21. El-Kalay MA, Koochaki Z, Schutz PW, Gaylor JD. Efficient continuous flow washing of red blood cells for exogenous agent loading using a hollow fiber plasma separator. Artif. Organs. 1989; 13 (6): 515– 524.
22. Ihler GM, Glew RH, Schunure FW. Enzyme loading of erythrocytes. Proc. Natl. Acad. 1973; 70: 663– 2666.
23. Dale G, Green R, Widder KJ. Methods in Enzymology. vol. 149, Academic Press, San Diego, 1987, pp. 229– 234.
24. Magnani M, Rossi L, Brandi G, Schiavano GF, Montroni M, Piedimonte G. Targeting antiretroviral nucleoside analogues in phosphorylated form to macrophages: in vitro and in vivo studies. Proc. Natl. Acad. Sci. U. S. A. 1992; 89 (14): 6477– 6481.
25. Gardos G. Akkumulation de Kalium Onen Durch Menschiche Blutkorperchen. Acta Physiol. Acad. Sci. Hung. 1953; 6: 191–196.
26. Iher GM, Glew RM, Schnure FW. Enzyme Loading of Erythrocytes. Proc. Natl. Acad. Sci. USA. 1973; 70: 2663–2666.
27. Zimmermann U. Jahresbericht der Kernforschungsanlage Julich GmbH. Nuclear Research Center, Julich, 1973, pp. 55–58.
28. Lewis DA, Alpar HO. Therapeutic possibilities of drugs encapsulated in erythrocytes. Int. J. Pharm. 1984; 22: 137–146.
29. Zimmermann U. Cellular drug-carrier systems and their possible targeting, in: E.P. Goldberg (Ed.), Targeted Drugs. John Wiley & Sons, New York, 1983, pp. 153–200.
30. Jaitely V, Kanaujia P, Venkatesan N, Jain S, Vyas SP. Resealed erythrocytes: drug carrier potentials and biomedical applications. Indian Drugs. 1996; 33: 589–594.
31. Jain S, Jain NK. Engineered erythrocytes as a drug delivery system. Indian J. Pharm. Sci. 1997; 59: 275–281.
32. Updike SJ, Wakamiya RT. Infusion of red blood cell-loaded asparaginase in monkey. J. Lab. Clin. Med. 1983; 101: 679–691.
33. Alpar HO, Irwin WJ. Some unique applications of erythrocytes as carrier systems. Adv. Biosci. 1987; 67: 1–9.
34. Eichler HC, Gasic S, Daum B, Bacher S, Sreger G. In vitro drug release from human carrier erythrocytes. Adv. Biosci. 1987; 67: 11–15.
35. Summers MP. Recent advances in drug delivery. Pharm. J. 1983; 230: 643–645.
36. Talwar N, Jain NK. Erythrocytes as carrier of primaquin preparation: characterization and evaluation. J. Control. Release. 1992; 20: 133–142.
37. Lewis DA. Red blood cells for drug delivery. Pharm. J. 1984; 233: 384–385.
38. Adriaenssens K, Karcher D, Lowenthal A, Terheggen HG. Use of enzyme-loaded erythrocytes in in-vitro correction of arginase-deficient erythrocytes in familiar hyperargininemia. Clin. Chem. 1976; 22: 323–326.
39. Sprandel U. Towards cellular drug targeting and controlled release of drugs by magnetic fields. Adv. Biosci. 1987; 67: 243–250.
40. Jenner DJ, Lewis DA, Pitt E, Offord RA. The effect of the intravenous administration of corticosteroids encapsulated in intact erythrocytes on adjuvant arthritis in the rat. Br. J. Pharmacol. 1981; 73: 212–213.
41. Kinosita K, Tsong TY. Survival of sucrose-loaded erythrocytes in the circulation. Nature 1978; 272: 258–260.
42. Guyton AC, Hall JE. Red blood cells, anemia and polycytemia, Textbook of Medical Physiology. W. B. Saunders, Philadelphia, 1996, pp. 425–433.
43. Alpar HO, Lewis DA. Therapeutic efficacy of asparaginase encapsulated in intact erythrocytes. Biochem. Pharmacol. 1985; 34: 257–261.
44. Erchler HG, Gasic S, Bauer K, Korn A, Bacher S. In vivo clearance of antibody-sensitized human drug carrier erythrocytes. Clin. Pharmacol. Ther. 1986; 40: 300–303.
45. Baker R. Entry of ferritin into human red cells during hypotonic haemolysis. Nature. 1967; 215: 424–425.
46. Ihler GM, Tsong HCW. Hypotonic hemolysis methods for entrapment of agents in resealed erythrocytes. Methods Enzymol. 1987; 149: 221–229.
47. Vienken J, Jeltsch E, Zimmermann U. Penetration and entrapment of large particles in erythrocytes by electrical breakdown techniques. Cytobiologie. 1978; 17: 182–186.
48. Jain S, Jain SK, Dixit VK. Erythrocytes based delivery of isoniazid: preparation and in vitro characterization. Indian Drugs. 1995; 32: 471–476.
49. Pitt E, Lewis DA, Offord R. The use of corticosteroids encapsulated in erythrocytes in the treatment of adjuvant induced arthritis in the rat. Biochem. Pharmacol. 1983; 132: 3355–3358.
50. Lynch WE, Sartiano GP, Chaffar A. Erytrocytes as carriers of chemotherapeutic agents for targeting the reticuloendothelial system. Am. J. Hematol. 1980; 9 (3): 249– 259.
51. Jain S, Jain NK. Engineered erythrocytes as a drug delivery system. Indian J. Pharm. Sci. 1997; 59: 275–281.
52. Moss HA, Tebbs SE, Faroqui MH, Herbst T, Isaac JL, Brown J, Elliott TS. A central venous catheter coated with benzalkonium chloride for the prevention of catheter-related microbial colonization. Eur. J. Anaesthesiol. 2000; 17 (11): 680– 687.
53. Valbonesi M, Bruni R, Florio G, Zanella A, Bunkens H. Cellular contamination of plasma collected with various apheresis systems. Transfus. Apher. Sci. 2001; 24: 91–94.
54. Sugai Y, Sugai K, Fuse A. Current status of bacterial contamination of autologous blood for transfusion. Transfus. Apher. Sci. 2001; 24: 255– 259.
55. lvarez FEA, Lichtiger B. Bacterial contamination of cellular blood components. Curr. Issues in Transfus. Med. 1995; 3 (3): 46.
56. Benatti U, Zochhl E, Tonetti M, Guida L, DeFlora A. Comparative tissue distribution and metabolism of free versus erythrocyte-encapsulated adriamycin in mouse. Adv. Biosci. 1987; 67: 129–136.
57. Ihler GM. Erythrocyte carriers. Pharmacol. Ther. 1983; 20: 151–169.
58. Updike SJ, Wakarniya RT, Lightfoot EN. Asparaginase entrapped in red blood cells: action and survival. Science. 1976; 193: 681–683.
59. Talwar N, Jain NK. Erythrocytes as carrier of metronidazole: in vitro characterization. Drug Devel. Ind. Pharm. 1992; 18: 1799–1812.
60. Pitt E, Johnson CM, Lewis DA, Jenner DA, Offord R. Encapsulation of drugs in intact erythrocytes: an intravenous delivery system. Biochem. Pharmacol. 1983; 22: 3359–3368.
61. Iher GM, Glew RM, Schnure FW. Enzyme loading of erythrocytes. Proc. Natl. Acad. Sci. U. S. A. 1973; 70: 2663–2666.

62. Deloach JR, Ihler GM. A dialysis procedure for loading of erythrocytes with enzymes and lipids. Biochim. Biophys. Acta. 1977; 496: 136–145.
63. Bhaskaran S, Dhir SS. Resealed erythrocytes as carriers of Salbutamol sulphate. Indian J. Pharm. Sci. 1995; 57: 240–242.
64. Kravtozoff R, Ropars C, Laguerre M, Muh JP, Chassaigne M. Erythrocytes as carriers for L-asparaginase: methodological and mouse in-vivo studies. J. Pharm. Pharmacol. 1990; 42: 473–476.
65. Berman JD. Antileishmanial activity of red cell encapsulated drugs. Adv. Biosci. 1987; 67: 145–152.
66. Deloach JR, Andrews K, Sheffield CL, Koths K. Subcutaneous administration of [35-S] r-IL-2 in mice carrier erythrocytes: alteration of IL-2 pharmacokinetics. Adv. Biosci. 1987; 67: 183–190.
67. Jrade M, Villereal MC, Boynard M, Ropars C. Rheological approach to human red blood cell carriers' desferrioxamine encapsulation. Adv. Biosci. 1987; 67: 29–36.
68. Zanella A, Rossi F, Sabionedda L, Russo V. Desferrioxamine loading of red cells for transfusion. Adv. Biosci. 1987; 67: 17–27.
69. Fiorelli G, Fargion S, Piperno A, Cappellini MD, Rossi F, Sabionedda L, Zanella A. Transfusion of thalasemic patients with desferrioxamine loaded standard red blood cell units. Adv. Biosci. 1987; 67: 47–54.
70. Jain S, Jain SK, Dixit VK. Magnetically guided rat erythrocytes bearing isoniazid: preparation, characterization, and evaluation. Drug Devel. Ind. Pharm. 1997; 23: 999–1006.
71. Tajerzadeh H, Hamidi M. Evaluation of the hypotonic preswelling method for encapsulation of enalaprilat in human intact erythrocytes. Drug Devel. Ind. Pharm. 2000; 26: 1247–1257.
72. Hamidi M, Tajerzadeh H, Rouini MR, Dehpour AR. In vitro characterization of human intact erythrocytes loaded by enalaprilat. Drug Deliv. 2001; 8: 231–237.
73. Bird J, Best R, Lewis DA. The encapsulation of insulin in erythrocytes. J. Pharm. Pharmacol. 1983; 35: 246–247.
74. Franco R, Barker R, Weiner M. The nature and kinetics of red cell membrane changes during the osmotic pulse method of incorporating xenobiotics into viable red cells. Adv. Biosci. 1987; 67: 63–72.
75. Deuticke B, Kim M, Zolinev C. The influence of amphotericin-B on the permeability of mammalian erythrocytes to non-electrolytes, anions and cations. Biochim. Biophys. Acta 1973; 318: 345–359.
76. Kruse CA, James GT, Freehauf CL, Williams CM. Methotrexate loaded erythrocytes carriers: optimization their formation, their characterization, and their pharmacological efficiency in treating hepatoma 129 ascites tumors in mice. Adv. Biosci. 1987; 67: 137–144.
77. Mitchell DH, James GT, Kruse CA. Bioactivity of electric fieldpulsed human recombinant interleukin-2 and its encapsulation into erythrocyte carriers. Biotechnol. Appl. Biochem. 1990; 12 (3): 264–275.
78. Mulholland SE, Lee S, McAuliffe DJ, Doukas AG. Cell loading with laser-generated stress waves: the role of the stress gradient. Pharm. Res. 1999; 16 (4): 514–518.
79. Lotero LA, Olmos G, Diez JC. Delivery to macrophages and toxic action of etoposide carried in mouse red blood cells. Biochim. Biophys. Acta 2003; 1620 (1–3): 160–166.
80. Ihler GM, Tsang HCW. Hypotonic Hemolysis Methods for Entrapment of Agents in Resealed Erythrocytes. Methods Enzymol. 1987; 149: 221– 229.
81. Deloach JR, Harris RL, Ihler GM. An Erythrocyte Encapsulator Dialyzer Used in Preparing Large Quantities of Erythrocyte Ghosts and Encapsulation of a Pesticide in Erythrocyte Ghosts. Anal. Biochem. 1980; 102: 220–227.
82. Ihler GM. Erythrocyte Carriers. Pharmacol. Ther. 1983; 20: 151–169.
83. Magnani M. Biotechnol. Appl. Biochem. 1998; 28: 1–6.
84. Jaitely V. Resealed Erythrocytes: Drug Carrier Potentials and Biomedical Applications. Indian Drugs. 1996; 33: 589–594.
85. Pitt E. Encapsulation of Drugs in Intact Erythrocytes: An Intravenous Delivery System. Biochem. Pharmacol. 1983; 22: 3359–3368.
86. Deloach JR, Ihler GM. A Dialysis Procedure for Loading of Erythrocytes with Enzymes and Lipids. Biochim. Biophys. Acta. 1977; 496: 136–145.
87. Talwar N, Jain NK. Erythrocytes as Carriers of Metronidazole: In-Vitro Characterization. Drug Dev. Ind. Pharm. 1992; 18: 1799–1812.
88. Lewis DA. Red Blood Cells for Drug Delivery. Pharm. J. 1984; 233: 384–385.
89. Baker R. Entry of Ferritin into Human Red Cells during Hypotonic Hemolysis. Nature 1967; 215: 424-425.
90. Updike SJ, Wakamiya RT. Infusion of Red Blood Cell-Loaded Asparaginase in Monkey. J. Lab. Clin.Med. 1983; 101: 679–691.
91. Updike SJ, Wakarniya RT, Lightfoot EN. Asparaginase Entrapped in Red Blood Cells: Action and Survival Science. 1976; 193: 681–683.
92. Adriaenssens K. Use of Enzyme-Loaded Erythrocytes in In Vitro Correction of Arginase Deficient Erythrocytes in Familiar Hyperargininemia. Clin. Chem. 1976; 22: 323–326.
93. Bhaskaran S, Dhir SS. Resealed Erythrocytes as Carriers of Salbutamol Sulphate. Indian J. Pharm. Sci. 1995; 57: 240–242.
94. Rechsteiner MC. Uptake of Protein by Red Cells. Exp. Cell Res. 1975; 43: 487–492.
95. Field WN, Gamble MD, Lewis DA. A Comparison of Treatment of Thyroidectomized Rats with Free Thyroxin and Thyroxin Encapsulated in Erythrocytes. Int. J. Pharm. 1989; 51: 175–178.
96. Alpar HO, Irwin WJ. Some Unique Applications of Erythrocytes as Carrier Systems. Adv. Biosci. 1987; 67: 1–9.
97. Alpar HO, Lewis DA. Therapeutic Efficacy of Asparaginase Encapsulated in Intact Erythrocytes. Biochem. Pharmacol. 1985; 34: 257–261.
98. Pitt E, Lewis DA, Offord R. The Use of Corticosteroids Encapsulated in Erythrocytes in the Treatment of Adjuvant Induced Arthritis in the Rat. Biochem. Pharmacol. 1983; 132: 3355–3358.
99. Bird J, Best R, Lewis DA. The Encapsulation of Insulin in Erythrocytes. J. Pharm. Pharmacol. 1983; 35: 246–247.
100. Tajerzadeh H, Hamidi M. Evaluation of the Hypotonic Preswelling Method for Encapsulation of Enalaprilat in Human Intact Erythrocytes. Drug Dev. Ind. Pharm. 2000; 26: 1247–1257.
101. Jain S, Jain SK, Dixit VK. Magnetically Guided Rat Erythrocytes Bearing Isoniazid: Preparation, Characterization, and Evaluation. Drug Dev. Ind. Pharm. 1997; 23: 999–1006.
102. Klibansky C. PhD, thesis. Hebrew University, Jerusalem, Israel, 1959.
103. Dale GL, Villacorte DG, Beutler E. High Yield Entrapment of Protein into Erythrocytes. Biochem.Med. 1977; 18: 220–225.
104. Benatti U. Comparative Tissue Distribution and Metabolism of Free Versus Erythrocyte-Encapsulated Adriamycin in the Mouse. Adv. Biosci. 1987; 67: 129–136.
105. Kravtozoff R. Erythrocytes as Carriers for L-Asparaginase: Methodological and Mouse In-Vivo Studies. J. Pharm. Pharmacol. 1990; 42: 473–476.
106. Jrade M. Rheological Approach to Human Red Blood Cell Carriers Desferrioxamine Encapsulation. Adv. Biosci. 1987; 67: 29–36.
107. Berman JD. Antileishmanial Activity of Red Cell Encapsulated Drugs. Adv. Biosci. 1987; 67: 145–152.
108. Deloach JR. Subcutaneous Administration of [35-S] r-IL-2 in Mice Carrier Erythrocytes: Alteration of IL-2 Pharmacokinetics. Adv. Biosci. 1987; 67: 183–190.
109. Deloach JR, Doleskey R. Preparation and Properties of Microcytic Carrier Erythrocytes from Sheep and Goats. Adv. Biosci. 1987; 67: 199–212.
110. Zanella A. Desferrioxamine Loading of Red Cells for Transfusion. Adv. Biosci. 1987; 67: 17–27.
111. Fiorelli G. Transfusion of Thalasemic Patients with Desferrioxamine Loaded Standard Red Blood Cell Units. Adv. Biosci. 1987; 67: 47–54.
112. Villareal MC. Approach to Optimization of Inositol Hexaphosphate Entrapment into Human Red Blood Cells. Adv. Biosci. 1987; 67: 55–62.
113. Hurel C. Optimization of Desferrioxamine Loading in Red Blood Cells. Adv. Biosci. 1987; 67: 37–46.
114. Villareal MC. Modification of Cardiac Parameters in Piglets after Infusion of IHP-Loaded Red Blood Cells. Adv. Biosci. 1987; 67: 81–88.
115. Teisseire B. In Vivo Consequences of Rightward Shift of the Hemoglobin Dissociation Curve. Adv. Biosci. 1987; 67: 89–94.
116. Garin MI. Erythrocytes as Carriers for Recombinant Human Erythropoietin. Pharm. Res. 1996; 13: 869–874.
117. Davson H, Danielli JF. Dannen Conn. Hanfer Publishing Co., Germany, 1970, pp. 80.
118. Billah MM. Permeability Characteristics of Erythrocyte Ghosts Prepared Under Isoionic Conditions by a Glycol-Induced Osmotic Lysis. Biochim Biophys Acta. 1977; 465 (3): 515–526.
119. Bird J, Best R, Lewis DA. The Encapsulation of Insulin in Erythrocytes. J. Pharm. Pharmacol. 1983; 35: 246–247.

120. Deuticke B, Kim M, Zolinev C. The Influence of Amphotericin- B on the Permeability of Mammalian Erythrocytes to Nonelectrolytes, anions and Cations. Biochim. Biophys. Acta. 1973; 318: 345–359.
121. Kitao T, Hattori K, Takeshita M. Agglutination of Leukemic Cells and Daunomycin Entrapped Erythrocytes with Lectin In Vitro and In Vivo. Experimentia. 1978; 341: 94–95.
122. Lin W. Nuclear Magnetic Resonance and Oxygen Affinity Study of Cesium Binding in Human Erythrocytes. Arch Biochem Biophys. 1999; 369 (1): 78–88.
123. Kinosita K, Tsong TY. Hemolysis of Human Erythrocytes by a Transient Electric Field. Proc. Natl. Acad. Sci. USA. 1977; 74: 1923-1927.
124. Zimmermann U, Riemann F, Pilwat G. Enzyme Loading of Electrically Homogenous Human Red Blood Cell Ghosts Prepared by Dielectric Breakdown. Biochim. Biophys. Acta. 1976; 436: 460–474.
125. Tsong TY, Kinosita K. Use of Voltage Pulses for the Pore Opening and Drug Loading, and the Subsequent Resealing of Red Blood Cells. Bibl Haematol. 1985; 51: 108–114.
126. Mitchell DH, James GT, Kruse CA. Bioactivity of Electric Field-Pulsed Human Recombinant Interleukin-2 and Its Encapsulation into Erythrocyte Carriers. Biotechnol. Appl. Biochem. 1990; 12 (3): 264–275.
127. Mouneimne Y. Electro-Insertion of Xeno-Glycophorin into the Red Blood Cell Membrane. Biochem. Biophys. Res. Commun. 1989; 159 (1): 34–40.
128. Mangal PC, Kaur A. Electroporation of Red Blood Cell Membrane and its Use as a Drug Carrier System. Ind. J. Biochem. Biophys. 1991; 28 (3): 219-221.
129. Schrier SL. Energized Endocytosis in Human Erythrocyte Ghosts. J. Clin. Invest. 1975; 56 (1): 8–22.
130. Schrier SL. Shape Changes and Deformability in Human Erythrocyte Membranes. J. Lab. Clin.Med. 1987; 110 (6): 791–797.
131. DeLoach J. R. Encapsulation of Exogenous Agents in Erythrocytes and the Circulating Survival of Carrier Erythrocytes. J. Appl. Biochem. 1983; 5 (3): 149–157.
132. Li LH. Electrofusion Between Heterogeneous-Sized Mammalian Cells in a Pellet: Potential Applications in Drug Delivery and Hybridoma Formation. Biophys J. 1996; 71 (1): 479–486.
133. Nicolau C, Gersonde K. Incorporation of Inositol Hexaphosphate into Intact Red Blood Cells, I: Fusion of Effector-Containing Lipid Vesicles with Erythrocytes. Naturwissenschaften. 1979; 66 (11): 563–566.
134. Luque J, Garı´n MI, Sanz S, Ropero P, Pinilla M. The Use of Resealed Erythrocytes as Carriers and Biorreactors, Advances in Experimental Medicine and Biology. vol. 326, 1992, pp. 81–89.
135. Talwar N, Jain NK. Erythrocytes as carriers of metronidazole: in vitro characterization. Drug Dev. Ind. Pharm. (1992); 18 (16): 1799– 1812.
136. Sanz S, Lizano C, Luque J, Pinilla M. In vitro and in vivo study of glutamate dehydrogenase encapsulated into mouse erythrocytes by hypotonic dialysis procedure. Life Sci. 1999; 65 (26): 2781– 2789.
137. DeLoach JR, Peters S, Pinkard O, Glew R, Ihler G. Effect of glutaraldehyde on enzyme-loaded erythrocytes. Biochim. Biophys. Acta. 1977; 496: 507–515.
138. Noel-Hocquet S, Jabbouri S, Lazar S, Maunier JC, Guillaumet G, Ropars C, Magnani M, DeLoach JR. The Use of Resealed Erythrocytes as Carriers and Biorreactors, Advances in Experimental Medicine and Biology. vol. 326, 1992, pp. 215-221.
139. Flora AD, Benatti U, Guida L, Zocchi E. Encapsulation of Adriamycin in human erythrocytes. Proc. Natl. Acad. Sci. 1986; 83: 7029-7033.
140. Pitt E, Johnson CM, Lewis DA. Encapsulation of drugs in intact erythrocytes: an intravenous delivery system. Biochem. Pharmacol. 1983; 32 (22): 3359-3368.
141. Eichler HC, Gasic S, Daum B, Bacher S, Steger G. In vitro drug release from human carrier erythrocytes. Adv. Biosci. 1987; 67: 11-15.
142. Hamidi M, Tajerzadeh H. Carrier erythrocytes: an overview. Drug Deliv. 2003; 10: 9-20.
143. Krantz A. Red-cell mediated therapy: opportunities and challenges. Blood Cells Mol. Diseases. 1997; 23 (3): 58-68.
144. Ihler GM, Glew RH, Schunure FW. Enzyme loading of erythrocytes. Proc. Natl. Acad. Sci. U. S. A. 1973; 70: 2663– 2666.
145. Garin MI, Lo´pez RM, Sanz S, Pinilla M, Luque J. Erythrocytes as carriers for recombinant human erythropoietin. Pharm. Res. 1996; 13 (6): 869-874.
146. Hamidi M, Tajerzadeh H, Dehpour AR, Rouini MR, Ejtemaee-Mehr S. In vitro characterization of human intact erythrocytes loaded by enalaprilat. Drug Deliv. 2001; 8 (4): 223-230.
147. Ito Y, Ogiso T, Iwaki M, Yoneda I, Okuda Y. In vitro stability of insulin-loaded erythrocytes after freezing storage. J. Pharmacobio-Dyn. 1989; 12: 201-207.
148. Rossi L, Serafini S, Cappellacci L, Balestra E, Brandi G, Schiviano GF, Franchetti P, Grifantini M, Perno CF, Magnani M. Erythrocyte-mediated delivery of a new homodinucleotide active against human immunodeficiency virus and herpes simplex virus. J. Antimicrob. Chemother. 2001; 47: 819-827.
149. Lizano C, Sanz S, Luque J, Pinilla M. In vitro study of alcohol dehydrogenase and acetaldehyde dehydrogenase encapsulated into human erythrocytes by an electroporation procedure. Biochim. Biophys. Acta. 1998; 1425 (2): 328-336.