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Resealed Erythrocytes: As a Specified tool in Novel Drug Delivery Carrier System

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ABSTRACT

Now a day's most of the investigations are focused only on the development of various drug delivery systems for targeting of the desired tissue to achieving required therapeutic concentration with less amount of dose. Targeting of an active biomolecule from efficient drug delivery where pharmacological agent directed specifically to its target site can be approached by either chemical modification or by appropriate carrier. Here erythrocytes have proved that they have potential carrier capabilities. Such drug-loaded carrier erythrocytes are biocompatible and biodegradable, and possess very long circulation half lives. And which 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, which are called as resealed erythrocytes. The present article gives information about morphology, isolation, properties, and methods of drug loading, characterization, mechanism, and applications of resealed erythrocytes.

Keywords: Resealed erythrocytes, Cellular carriers, entrapping drug, half lives.

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INTRODUCTION

Present pharmaceutical circumstances are designed at development of drug delivery systems which make best use of the drug targeting along with high therapeutic benefits for safe and efficient treatment of diseases and also more patient compliance [3]. Here various carrier systems are present for targeting the particular tissue. The idea of a drug carrier system with target specificity is helpful for scientists and tremendous efforts have been made to achieve this goal**.** There are various carriers used for targeting drugs to various body tissues, the cellular carriers meet several criteria enviable in clinical applications, along with that most important being the biocompatibility of carrier and its degradation products. Leucocytes, platelets, erythrocytes, nanoerythrocytes, hepatocytes, and fibroblasts etc. have been proposed as cellular carrier systems[2]. Among those Resealed erythrocytes are gaining more popularity as targeted drug carriers, due to their ability to circulate throughout the body, biocompatibility, zero order release kinetics, reproducibility and ease of preparation. [7]

Morphology and physiology of erythrocytes:

Erythrocytes are the most abundant cells in the human. These cells were described in human blood samples by Dutch Scientist Lee Van Hock in 1674. In the 19th century, Hope Seyler identified hemoglobin and its crucial role in oxygen delivery to various parts of the body. 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 _m3. 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 (Gopal et al., 2007). 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**. [1]

Composition of Erythrocytes:

Blood contains about 55% of fluid portion (plasma) 45% of corpuscles or formed elements. Normal blood cells have extensile, elastic, biconcave and non nucleated configuration with a diameter ranging from 6-9 µ and the thickness is nearly 1-2 µ.

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Erythrocytes have a solid content of about 35% most of which is Hb and rest 65% being water. Lipid content of erythrocytes includes cholesterol, lecithin and cephaelins.

Isolation of erythrocytes:

The cellular content is about 40-50% of the blood volume and contains erythrocytes (red blood cells, RBC), Leukocytes (while blood cells, WBC) and thrombocytes (platelets). The primarily water (90 to 92%) and protein(7%).Blood is withdrawn from cardiac/splenic puncture (in case of small animal) and through veins (in case large animals) into a syringe containing drop of anticoagulant .The whole blood is centrifuged at 2500 rpm for 5min at 4±4^{0}c in a refrigerated centrifuge. The serum and Buffy coats are carefully removed and packed cells washed 3 times with phosphate buffer saline (PBS pH 7.4).The washed Erythrocyte are diluted with PBS and stored at 4^{0}c until used. [7] Refer table 1.

Table 1: Isolation of erythrocytes:

Sr.no.	Species	Washing buffer	Centrifugal force(g)
1	Rabbit	10mmol KH_2P0_4 /$NaHPO_4$	500-1000
2	Dog	15mmol KH_2P0_4 /$NaHPO_4$	500-1000
3	Human	154mmolNaCl	<500
4	Mouse	10mmol KH_2P0_4 /$NaHPO_4$	100-500
5	Cow	10-15mmol KH_2P0_4 /$NaHPO_4$	1000
6	Horse	2 mmol$MgCl_2$, 10 glucose	1000
7	Sheep	10 mmol KH_2P0_4 /$NaHPO_4$	500-1000
8	Pig	10 mmolKH_2P0_4 /$NaHPO_4$	500-1000

Properties of resealed erythrocyte of novel drug delivery carriers: [8, 9, and 10]

1) The drug should be released at target site in a controlled manner.
2) It should be appropriate size, shape and should permit the passage through capillaries and Minimum leakage of drug should take place.
3) It should be biocompatible and should have minimum toxic effect.
4) It should possess the ability to carry a broad spectrum of drug.
5) It should possess specific physicochemical properties by which desired target size could be recognized.
6) The degradation product of the carriers system, after release of the drug at the selected site should be biocompatible. It should be physico -chemically compatible with drug.
7) The carrier system should have an appreciable stability during storage.

Requirement for encapsulation:-

- Variety of biologically active substance i.e. mol wt ranges from 5000-60,000dalton can be entrapped in erythrocytes.

- Non-polar molecule like tetracycline may be entrapped in bovine RBC in tetracycline HCl.
- Generally, this method used for only polar molecules. And in some cases we can see non polar molecules also entrapped.
- Hydrophobic molecules can be entrapped in erythrocyte by absorbing over other Molecules.
- The size of molecule entrapped is a key factor when the molecule is smaller than sucrose and larger than B-galactosidase.

Advantages of Erythrocytes as Drug Carriers:

- ❖ Their biocompatibility, particularly when autologous cells are used, hence no possibility of triggered immune response.
- ❖ Their biodegradability with no generation of toxic products.
- ❖ The considerably uniform size and shape of the carrier.
- ❖ Relatively inert intracellular environment.
- ❖ Prevention of degradation of the loaded drug from inactivation by endogenous chemicals.
- ❖ The wide variety of chemicals that can be entrapped.
- ❖ The modification of pharmacokinetic and pharmacodynamic parameters of drug. Attainment of steady-state plasma concentration decreases.
- ❖ Fluctuations in concentration protection of the organism against toxic effects of drugs.

Disadvantage:-

1) They have a limited potential as carrier to non-phagocyte target tissue.
2) Possibility of clumping of cells and dose dumping may be there.

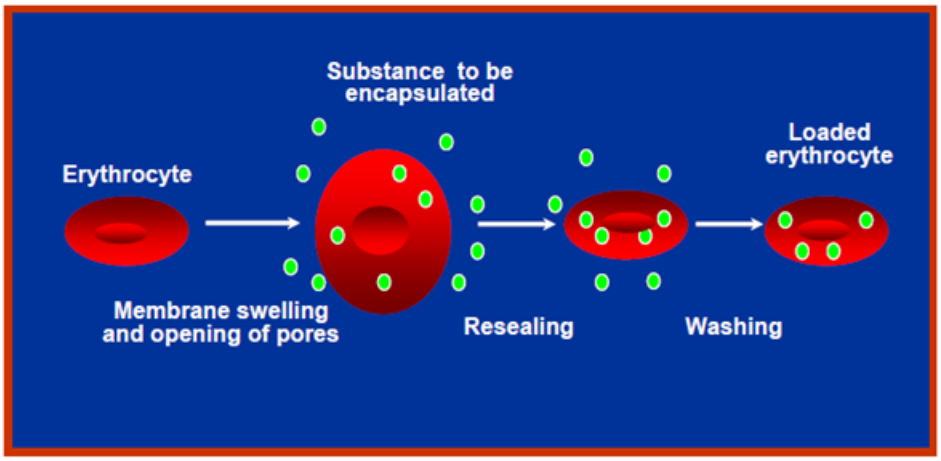

Fig 1: Schematic representation drug loading encapsulated erythrocytes [11]

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Method of drug loading in resealed erythrocytes:

There are several methods adopted for loading bioactive compounds in erythrocytes.

Physical osmosis based system

e.g., electrical pulse method

Chemical methods

e.g., chemical perturbation of the erythrocytes membrane

Irrespective of the method used for the drug loading, the optimal release of drug from erythrocyte membrane will depends on drug to have considerable degree of water solubility, lack of chemical or physical interaction with erythrocyte membrane, resistance against degradation with in erythrocytes, well defined pharmacodynamic and pharmacokinetic properties.

1. Hypo-osmotic lysis method:

In this process, the intracellular and extracellular solutes of erythrocytes are exchanged by osmotic lysis and resealing. The drug present will be encapsulated within the erythrocytes membrane by this process.

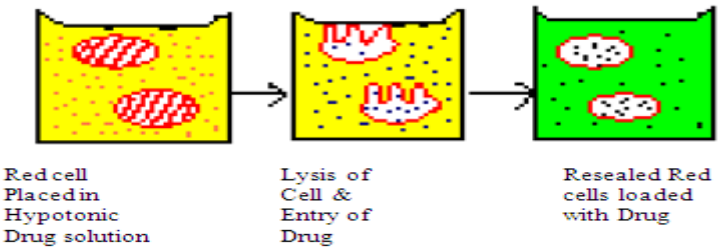

Fig 2: Drug loaded by Hypo-osmotic lysis method [7]

Hypotonic hemolysis:

This method is based upon hypotonic lysis of cells in solution containing the drug/enzyme to be entrapped followed by restoration of tonicity to reseal them.

Three type's ghosts can be distinguished

Type 1: Ghosts which reseal immediately after hemolysis
Type 2: Ghosts which reseal after, reversal of hemolysis by addition of alkali ions
Type 3: Ghosts which remain leaky under different experimental conditions

Erythrocytes have an exceptional capability for reversible shape changes.

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Hypotonic dilution:

This method was first investigated for the encapsulation of chemicals into erythrocytes. In this method a volume of packed erythrocytes is diluted in 2-20 volumes of aqueous solution of drug i.e. erythrocyte cells is placed in hypotonic solution (0.4% NaCl solution). The solution tonicity is then replaced by adding hypertonic buffer, we can get isotonic solution. The resultant mixture is then centrifuged the supernatant was discarded and the pellet is washed with isotonic buffer solution.

- Major drawbacks of this system are low entrapment efficiency, considerable loss of hemoglobin and other cell components.
- By these method enzymes such as ß-galactosides, ß-glucosides, asparginase, arginase and bronchodilator salbutamol successfully loaded.
- Efficiency of this method is 1-8%

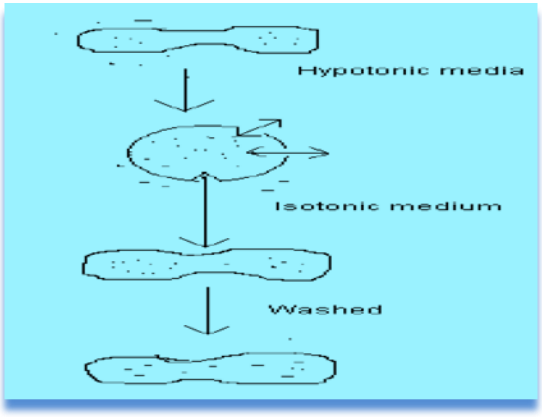

Fig 3: drug loaded by hypotonic dilution method [8]

Hypotonic dialysis:

This method was first reported by Kilbansky for loading enzymes and lipids in erythrocyte cells. In this process an isotonic buffered suspension of erythrocytes which have a hemocrit value of 80% is prepared and placed in a dialysis bag which was immersed in 10-20 volumes of hypotonic buffer (phosphate buffer). This medium was rotated for 2hrs by using mechanical rotators. The tonicity of the dialysis tube was restored by simultaneous addition of calculated amount of hypertonic buffer to the surrounding medium. Drug is loaded by adding the drug to a dialysis bag after the stirring complete. After drug loading dialysis bag placed in resealing buffer with mechanical rotator 30 min 37 ^{0}C.

- Efficiency of this method is 30-45%

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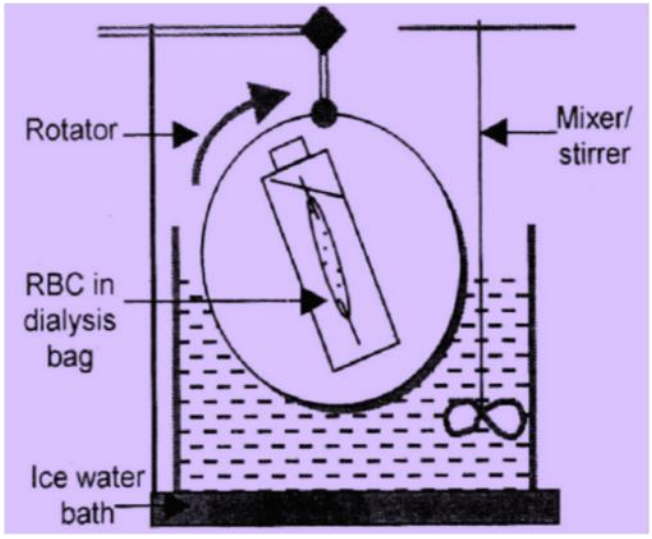

Fig 4: drug loaded by hypotonic dialysis method [24]

Hypotonic preswell technique:

This method was investigated by Rechsteiner in 1975 and this technique was modified by Jenner et al. for drug loading. This method is based on the following steps

1) Swelling: In this step erythrocyte cell swells without lysis when placed them in slightly hypotonic solution i.e. 0.6%w/v solution.

2) Drug Loading: After swelling relatively small volumes of aqueous drug solutions (loaded buffer) are added to the point of lysis. The slow swelling of cells resulted in good retention of the cytoplasmic constituents and incubated it for 5min at 0^0 C

3) Resealing: Drug loaded erythrocyte is placed in resealed buffer and incubated at 25^0 C.

This method is simple and faster than other methods causing minimum damage to cells. The drugs like Propranolol, asparginase, methotrexate, insulin, metronidazole, enalaprilat and isoniazid encapsulated by using this method.

- This method is 72% efficient.

Isotonic osmotic lysis:

This method is also known as the osmotic pulse method. It involves isotonic hemolysis. Erythrocytes are incubated in solutions of a substance having high membrane permeability; the solute will diffuse into the Erythrocyte cells because of the concentration gradient. Chemicals such as urea solution, polyethylene glycol, polypropylene and ammonium chloride have been used for isotonic hemolysis [24].

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Membrane perturbation by chemical agent:

This method is based upon 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. In 1980, this method was used successfully by Kitao and Hattori to entrap the antineoplastic drug daunomycin in human and mouse erythrocytes. However, these methods lead to irreversible destructive changes in the cell membrane and hence are not very popular.

Electro-insertion or electro encapsulation:

This method also called as electroporation; In 1973 Zimmermann tried an electrical pulse method to encapsulate bioactive compounds. This method is based on 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. The erythrocyte membrane is opened by a dielectric breakdown. Subsequently, the pores can be resealed by incubation at 37^0C in an isotonic medium. This dielectric breakdown can be achieved by polarization of erythrocyte membrane for 20µseconds using varied voltage of 2kv/cm. The potential difference across the membrane can be built up by either directly by inter and intracellular electrodes or indirectly by applying internal electric field to the cells. The extent of pore formation depends upon the electric field strength, pulse duration and ionic strength of suspending medium.

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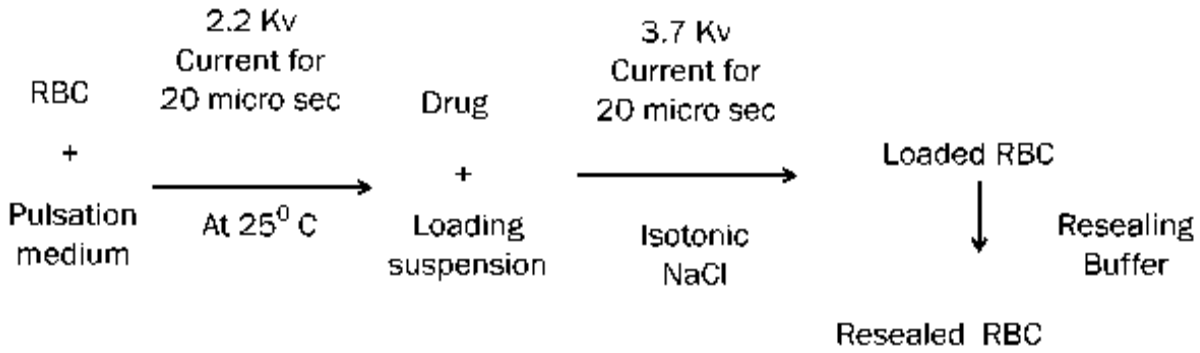
Here large molecules (like bovine serum albumin) and ribonucleases can be loaded. These can be loaded in to the osmotic swelling of electrically perforated erythrocytes.



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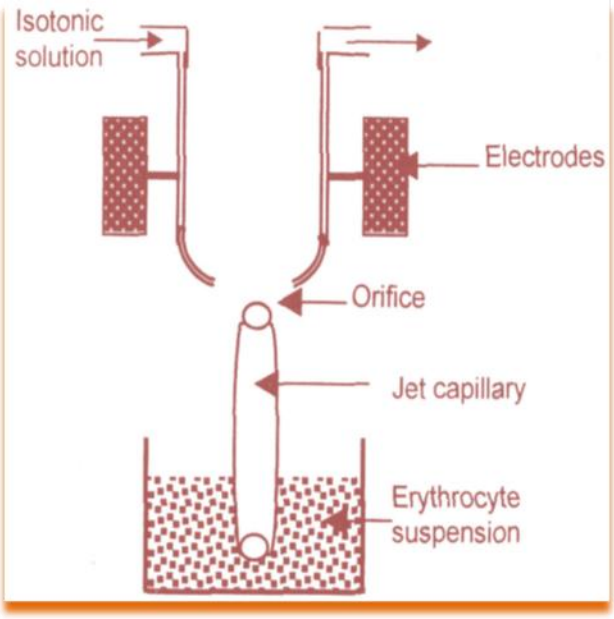

Fig 5: drug loaded by Electro encapsulation technique [9]

Entrapment by endocytosis:

This technique was reported by Schrier et al. in 1975. This technique involves the addition of one volume of packed erythrocytes to 9 volumes of buffer which contains ATP, $MgCl_2$, and CaCl2 and incubated it at 25^0C for 2 min.

Resealing of erythrocyte membrane by the addition of NaCl to 154 Mm followed by incubation for 2 min at 37^0C. These resealed erythrocytes are washed in 5mM imidazoleglycylglcine buffer, pH 7.4 containing 154mM NaCl.

Entrapment of the material by allowing endocytosis following incubation of washed resealed cell with buffer containing material to be entrapped for 30 min at 37^0C.

The drugs like primaquine, vinblastine, 8-amino-quinolines, chlorpromazine and related phenothiazines, hydrocortisone and tetracaine can be successfully entrapped by this technique.

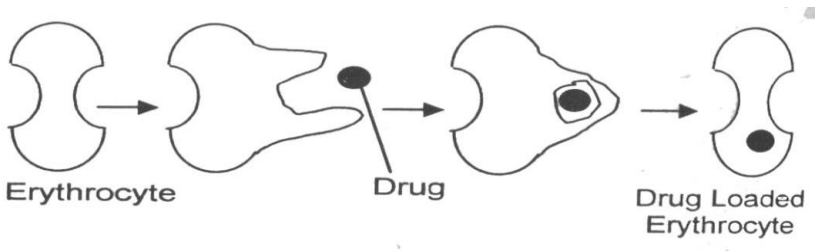

Fig 6: Drug loaded by entrapment endocytosis

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 [46, 47]. 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.

Lipid fusion technique:

In this method fused lipid vesicle containing bioactive molecule along with human erythrocytes leading to exchange of lipid entrapped drug molecule.

This technique is used for loading of inositol hexaphosphate into erythrocytes for the increased oxygen carrying capacity.

- This method provides very low encapsulation efficiency i.e. 1%

Table 2: Methods of drug loading: [1]

Method	%Loading	Advantages	Disadvantages
Dilution method	1-8%	Fastest and simplest for low mol.Wt	Less Entrapment efficiency
Presswell dilution	20-70%	Good retention of cytoplasm constituents and good survival in vivo	----------
Isotonic osmotic lysis	-------	Better in vivo surveillance	Time consuming, Impermeable to large molecules
Dialysis	30-45	Good retention of cytoplasm and good survival in vivo	----------

Characterization of resealed erythrocytes:

1. Drug content determination [7]:

After centrifugation at 3000 rpm for a fixed time interval drug loaded erythrocyte cells are deproteinized with acetonitrile. The clear supernatant liquid is analyzed for drug content.

2. In-vitro drug release and Hb content [8]:

The erythrocyte cell suspensions which have hemocrit value 5% are stored at 4^0C in amber colored glass container. Clear supernatant are drawn using a hypodermic syringe equipped with 0.45 filter for fixed time intervals and deproteined using methanol and were estimated for drug content. After centrifugation supernatant of each sample is collected and assayed, %Hb release calculated by using formula.

% Hb release=A540 of sample-A540 of background A540 of 100% Hb.

Laser light scattering technique may also be used to evaluate haemoglobin content of individual resealed erythrocytes.

3. Percentage cell recovery [9]:

Percentage cell recovery can be determined by counting the number of intact cells per cubic mm of packed erythrocytes before and after loading of the bioactive compound with the help of haemocytometer.

4. Morphology [24]:

Phase contrast optical microscopy, transmission electron microscopy and scanning electron microscopy are the microscopic methods used to evaluate the shape, size and surface features of loaded erythrocytes.

5. Osmotic shock [24]:

1ml of 10%erythrocyte suspension was diluted with 5ml of water and centrifuged the above mixture at 3000rpm for 15minutes. The supernatant was estimated for % Hb release spectrophotometrically.

6. Turbulence shock [8]:

This parameter indicates the effects of shear force and pressure by which resealed erythrocyte formulation are injected on the integrity of the loaded cells.

In this drug loaded cells are passed through a 23 gauge hypodermic at a flow rate of 10 ml/min which is comparable to the flow rate of blood. It is followed by collecting of an aliquot and centrifugation sample is estimated. Drug loaded erythrocytes appears to be less resistant to turbulence, probably indicating destruction of cells upon shaking.

7. Determination of entrapped magnetite [7]:

Determination of the concentration of a particular metal element present in a sample can be determined by Atomic Absorption spectroscopy. To a fixed amount of magnetite bearing erythrocyte add the HCl and these contents are heated at 60^0C for 2 hours and then 20%w/v trichloroacetic acid is added. These contents were centrifuged and collect supernatant. From this supernatant determine magnetite concentration using absorption spectroscopy.

8. Erythrocyte sedimentation rate (ESR) [10]:

It is an estimate of stability of suspension of erythrocyte cells in plasma and is related to the number and size of the red cells and to relative concentration of plasma proteins like fibrinogen and α, β globulins. This test is performed by determining the rate of sedimentation of blood cells in a standard tube. Normal blood ESR is 0 to 15 mm/hr. higher rate is indication of active but obscure disease processes.

9. Miscellaneous [10]:

Resealed erythrocyte can also be characterized by cell sizes, mean cell volume, energy metabolism, lipid composition, membrane fluidity, rheological properties, and density gradient separation.

Route of administration:

Intra peritoneal injection reported that survival of cells in circulation was equivalent to the cells administered by i.v. injection .They reported that 25% of resealed cell remained in circulation for 14 days they also proposed this method of injection as a method for extra vascular targeting of RBCs to peritoneal macrophages. Subcutaneous route for slow release of entrapped agent's. They reported that the loaded cell released encapsulated molecules at the injection site.

Mechanism of release of loaded drugs:

There are mainly three ways for a drug release from the erythrocyte carriers

- Phagocytosis:

  By the process of phagocytosis normally erythrocyte cells removed from the blood circulation. The degree of cross linking determines whether liver or spleen will preferentially remove the cells.
- Diffusion through the membrane of the cells:

  Diffusion through the membrane depends on the drug molecule penetrate through a lipid bilayer i.e. bioactive compound have lipid solubility.
- Using a specific transport system.

  Most of the drug molecules enter cells by a specific membrane protein system because the carriers are proteins with many properties analogous to that of enzymes.

Applications in in-vivo:

Targeting of bioactive agents to RE system [7]:

Damaged erythrocytes are rapidly clear off from circulation by phagocytic cells spleen and liver. By modifying the erythrocytic membrane can therefore used to target the liver and spleen.

Various methods used to modify the surface characteristics of erythrocytes include surface modification with antibodies, damage by heat treatment, glutaradehyde, carbohydrates such as sialic acid, sulfhydryl. The drug encapsulated erythrocytes have been used for RBC targeting in the treatment of following diseases.

(V) Delivery of antiviral agents [8]:

Several reports have been cited in the literature about antiviral agents entrapped in resealed erythrocytes for effective delivery and targeting. Because most antiviral drugs are nucleotides or nucleoside analogs, their entrapment and exit through the membrane needs careful consideration. Nucleosides are rapidly transported across the membrane whereas nucleotides are not and thus exhibiting prolonged release profiles. The release of nucleotides requires conversion of these moieties to purine or pyrimidine bases. Resealed erythrocytes have been used to deliver deoxycytidine derivatives, recombinant herpes simplex virus type 1 (HSV-1) glycoprotein B, azidothymidine derivatives, azathioprene, acyclovir and fludarabine phosphate.

Table 3: Examples of delivery of antiviral agents: [8, 17, 30, 31]

Categories of Drugs	Name of Drugs	Purpose
Azidothymidine Derivative	Azidothymidine homodinucleotideloaded erythrocytes	Slow delivery of the antiretro viral drug azidothymidine
Deoxycytidine Derivatives	Antiviral nucleotide Analogues	Encapsulated into erythrocytes for targeting to macrophages
Azathioprene and Acyclovir Derivatives	Heterodinucleotide of azidothymidine and acyclovir	Selective delivery to macrophage for protection against Human Immunodeficiency Virus or Herpes Simplex Virus

(VI) **Improvement in oxygen delivery to tissues [10]:**

Hemoglobin is the protein responsible for the oxygen-carrying capacity of erythrocytes. Under normal conditions, 95% of hemoglobin is saturated with oxygen in the lungs, whereas under physiologic conditions in peripheral blood stream only 25% of oxygenated hemoglobin becomes deoxygenated. Thus, the major fraction of oxygen bound to hemoglobin is recirculated with venous blood to the lungs. The use of this bound fraction has been suggested for the treatment of oxygen deficiency. 2, 3-Diphosphoglycerate (2, 3-DPG) is a natural effector of hemoglobin. The binding affinity of hemoglobin for oxygen changes reversibly with changes in intracellular concentration of 2, 3-DPG. This compensates for

changes in the oxygen pressure outside of the body, as the affinity of 2, 3-DPG to oxygen is much higher than that of hemoglobin.

Application of resealed erythrocytes for oxygen supply under the following conditions.

High altitude conditions.
Small number of alveoli.
Increased resistance to oxygen diffusion in the lungs.
Reduction in oxygen transport capacity.
Liver mediated detoxification process.

Table 4: Resealed erythrocytes used in delivery of enzymes:

Name of Enzyme	Purpose
L-Asparaginase	For treatment of leukemia
Aminolevulinate dehydratase	To treat adolescent patient suffering from lead poisoning.

Table 5: Resealed erythrocytes used in delivery of proteins and macromolecules:

Name of Proteins	Purpose
Insulin	For its sustained release
Mycotoxins	For specific delivery of this highly toxic proteins to liver macrophages
recombinant human erythropoietin (rHuEpo)	A cellular sustained delivery system
Aspirin and ferromagnetic colloid	Prevention of thromboembolism.

Table 6: Various application of resealed erythrocytes: [ref no 25,26]

Application	Drug/Enzyme/Macromolecule	References
Enzyme deficiency replacement therapy	β -galactosidase,β-fructofuronodase, urease, glucose 6-phosphatedehydrogenase	Ihler et al 1973, 1975
Thrombolytic activity	Brinase, aspirin, heparin	Eicher et al 1986
Iron overload	Desferroxamine	Green 1985
Chemotherapy	Rubomycin, methotrexate, daunomycin, cytosine	Kitao and Hattori 1980
Immunotherapy	Human recombinant interleukin-2	Deloach et al 1991
Circulating bioreactor	Arginase, uriease, luciferase	Magnani et al 1992

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Table 7: Resealed erythrocytes used in other than RES organ targeting : [8]

Approaches	Type of Drugs	Objective/Purpose
Magnet-responsive Erythrocyte Ghosts	encapsulation of small paramagnetic particles into erythrocytes	Localization to a particular location under the influence of external magnetic field.
Photosensitized Erythrocytes	Methotrexate and photosensitized by subsequent exposure to a haematoporphyrin derivative	A combination of chemotherapy and photodynamic therapy could be a useful modality in the treatment of tumors of body located at site other than RES predominant organs. OR As a phototriggered carrier/delivery system for methotrexate in tumor therapy.
Antibody Anchored Erythrocytes (Immunoerythrocytes):	Antibody coating of resealed drug carrier	Drug targeting to the RES.
Ultrasound Mediated Delivery of Erythrocytes loaded drug(s):	Erythrocytes colloidal particles and red blood cells	Delivery to tissue through micro vessel ruptures created by targeted micro bubble destruction with ultrasound.

Table 8: Resealed erythrocytes used in RES targeting: [25,26,27,28]

Treatment/Diseases	Name of Drug(s)	Purpose
Treatment of lysosomal storage diseases	Lysosomal enzymes, C-glucuronidase, 13-galactosidase and 6-giucosidase	To deliver lysosomal enzymes and drugs to lysosomes of the erythrophagocytic cells.
Treatment of Gaucher's disease	Glucocerebrosidase	Loaded cells survived for 10 days in treated patient and no untoward reactions were found with respect to blood counts, blood pressure and renal functions.
Treatment of liver tumors	Anticancer like Bleomycin, Adriamycin, Carboplatin, Gentamycin, etc encapsulated in erythrocytes	Targeting to hepatic carcinomas.
Treatment of parasitic diseases	Pentamidine loaded, immunoglobulin-G coated erythrocytes Glutaraldehyde treated erythrocytes	Targeting of drugs in the treatment of parasitic diseases in which the parasite resides in the organs of RES.e.g. macrophage-contained leishmania. Liver targeting of an antimalarial agent - primaquine phosphate and an antiamoebic agent, metronidazole
Removal of RES iron overload	Desferoxamine, an iron-chelating drug in erythrocyte ghosts	To promote excretion of iron in patients with excess body stores.
Removal of Toxic Agents	Murine carrier erythrocytes containing bovine rhodanese and sodium thiosulphate	Antagonism of cyanide intoxication or to antagonize the lethal effects of potassium cyanide in mice.

Novel systems:

Nanoerythrosomes:

Nanoerythrosomes are vesicles prepared by the extrusion of RBC ghosts, the average diameter of these vesicles being 100nm. The process gave small vesicles with the size of liposomes. These spheroid particles were named 'nanoerythrosomes' and appear to be stable and maintain both the cytotoxic and antineoplastic activity of daunorubicin against mice leukemia P338D cells.(Jain and Jain,1996d ; Lejeune et al.,1994).7

Erythrosomes:

Erythrosomes are specially engineered vesicular systems in which chemically cross-linked human erythrocyte cytoskeletons are used as a support upon which a lipid bilayer (phosphatidyl choline) is coated.

These vesicles have been proposed as useful encapsulation system for macromolecular drugs.

CONLUSION

Now a day's there are numerous applications have been proposed for the use of resealed erythrocytes as carrier for drugs, enzyme replacement therapy etc. Until other carrier systems come of age, resealed erythrocytes technology will remain an active field for the further research. The use of resealed erythrocytes shows potential for a safe and effective delivery of various bioactive molecules for effective targeting. However, the concept needs further optimization to be converted into a regular drug delivery system. The coming years represent a significant time in this field as commercial applications are explored. In coming future, erythrocytes based delivery system with their capability to afford controlled and site specific drug delivery have been developed for disease management. For the present, it is concluded that erythrocyte carriers are "nano device in field of nanotechnology" considering their fabulous prospective.

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