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Review Article

Resealed Erythrocytes as a Carrier for Drug Targeting- A Review

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ABSTRACT

Good therapeutic action we can achieve by targeting to the site of drug delivery. To deliver the drug contents, most of the vesicular systems act as carriers. Among the carrier systems resealed erythrocytes are used as drug carriers because they possess great potential in drug targeting. Resealed erythrocytes have the ability to flow throughout the body, biocompatible, follows zero-order drug release mechanics, reliable, and easy to prepare the formulation. Most of the resealed erythrocytes used as drug carriers are quickly obsessed from the blood by macrophages of reticular endothelial system (RES), which are present in the liver, lung, and spleen of the body. Nowadays varied applications are projected for the utilization of resealed erythrocytes as a drug carrier, enzyme replacement therapy and targeting the drug to various sites etc.

1. Introduction

Erythrocytes also referred to as red blood cells (RBC), are extensively studied for his or her potential carrier capabilities for the delivery of medicine and drug-loaded microspheres. Such drug-loaded carrier erythrocytes are prepared simply by aggregation blood samples from the organism of interest, separating erythrocytes from plasma, entrapping drug among the erythrocytes, and resealing the resultant cellular carriers. Hence, these carriers are called resealed erythrocytes. The overall process is predicated on the response of those cells under osmotic conditions. Upon reinjection, the drug-loaded erythrocytes function as slow circulating depots and target the medicine to a system (RES). Blood contains different sorts of cells like erythrocytes (RBC), leucocytes (WBC), and platelets, among them erythrocytes are the foremost interesting carrier and possess great potential in drug delivery due to their ability to circulate throughout the body, zero-order kinetics, reproducibility, and ease of preparation1 primary aim for the event of this drug delivery system is to maximize therapeutic performance, reducing undesirable side effects of the drug also as increase patient compliance. Once within the reticuloendothelial system, the erythrocyte is attacked by liposomal enzymes that cause the breakage of the cellular membrane and therefore the degradation of the hemoglobin by the heme-oxygenase enzyme. Although the greater part of the destruction of the old erythrocytes occurs within the reticuloendothelial system, it's estimated that up to 10% of the loss of erythrocytes takes place in circulation Erythrocytes represent potential biocompatible vectors for numerous bioactive substances, together with medicine, enzymes, and proteins.

Anatomy, physiology, and composition of RBCs:

RBCs have shapes like biconcave discs with a diameter of 7.8 µm and a thickness close to 2.2 µm. Mature RBCs have a simple structure. It is also inelastic in nature. Their cell wall is both strong and versatile, which allows them to deform without rupturing as they squeeze through narrow capillaries. RBCs lack a nucleus and different organelles and should neither reproduce nor keep up intensive metabolic activities. RBCs are highly specialized for his or her oxygen transport function because their mature RBCs haven't any nucleus, all their internal space is out there for oxygen transport. Even the shape of RBC facilities its functions. The red blood corpuscle membrane, a dynamic, semi-permeable component of the cell, is related to energy metabolism within the maintenance of the permeability characteristic of the cell of varied cations (Na+, K++) and anions (Cl-HCO_3). Every RBC contains 280 million hemoglobin. A hemoglobin molecule consists of a protein called globin, composed of 4 polypeptide chains; a hoop-like non-protein pigment called heme is sure to each of the four chains. At the center of the haem, the ring combines reversibly with one oxygen molecule, permitting every hemoglobin molecule to bind four

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oxygen molecules. Composition of RBCs - water (63%), lipids (0.5), glucose (0.8%), mineral (0.7%), non-hemoglobin molecule (0.9%), and hemoglobin (33.67%). The structure of erythrocytes is shown in figure 1.

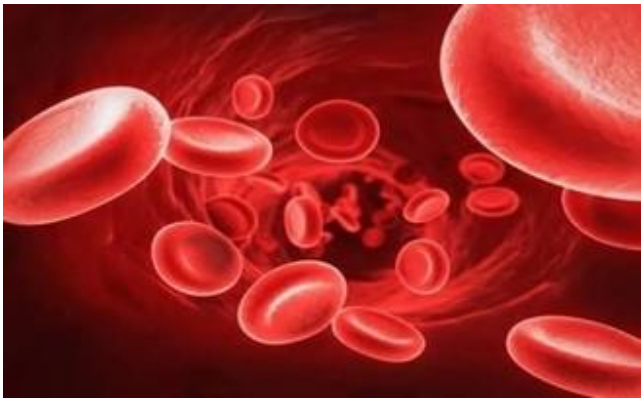


Figure 1: Structure of erythrocytes

Resealed Erythrocytes:

Drug-loaded carrier erythrocytes are prepared easily by collecting the blood samples from the organism of interest and erythrocytes are separated from plasma. Drug is entrapped in the erythrocytes and resealing the resultant cellular carriers [1]. Hence, these carriers are called resealed erythrocytes. The overall process is based on the response of these cells under osmotic conditions. The drug-loaded erythrocytes upon reinjection will serve as slow circulating depots and target the drugs to a reticuloendothelial system (RES) [2]. Schematic mechanism of resealed erythrocytes figure 2.

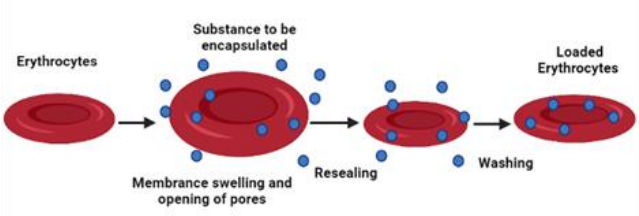


Figure 2: Schematic mechanism of resealed erythrocytes

Advantages of resealed erythrocytes:

- Since the erythrocytes are natural components of the body, they are biocompatible and biodegradable.
- These carriers are of uniform shape and size [3].
- Large quantities of drugs can be loaded within small cell volume thereby reducing the frequency of dosing.
- Prevents the degradation of the drugs by various hydrolytic enzymes or immune systems [4].
- A wide variety of drugs and other agents can be entrapped.
- Resealed erythrocytes have the ability to circulate throughout the body.
- They target the drugs to the organs of the reticuloendothelial system (RES).
- They protect the non-target tissues from the effects of the loaded drugs. E.g.: Anticancer drugs.
- The drugs can be entrapped without any chemical modification [5].
- Erythrocytes can be easily isolated and further processed due to the availability of several techniques.

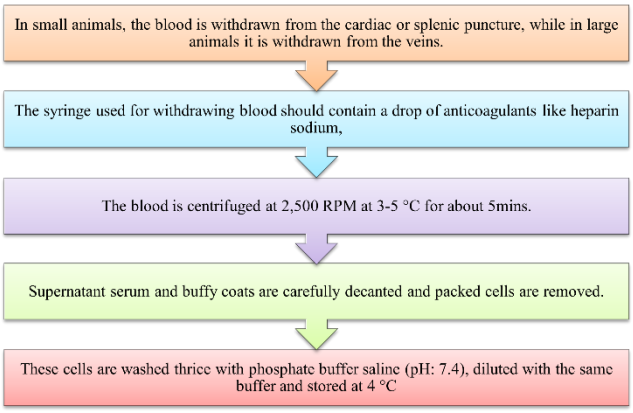
Disadvantages of resealed erythrocytes:

- Difficult to target drugs non- RES organs [6].
- Chances of clamping of the cells.
- Chances of dose dumping [7]

2. Isolation of Erythrocytes and Preparation of Resealed Erythrocytes:

Isolation of erythrocytes

The blood contains about 45% cellular components which are erythrocytes, leucocytes (white blood cells), and platelets. Erythrocytes are isolated from various animals such as mice, rats, chickens, rabbits, sheep, goats, dogs, monkeys, and even humans [8, 9].



Methods of Drug Loading (Entrapment Methods): [9, 10, 11, 12, 13, 14]

There are several methods used for loading the drugs into the erythrocytes. However, the efficacy of the drug entrapment depends on the properties of the drug. The drug to be entrapped should be possess the following properties,

1. High degree of water solubility.
2. Resistance towards degradation by the erythrocytes.
3. Non-interactive with the erythrocyte membrane.
4. Well-defined pharmacokinetic and pharmacodynamic properties.

The various methods of drug entrapment in erythrocytes have been described below.

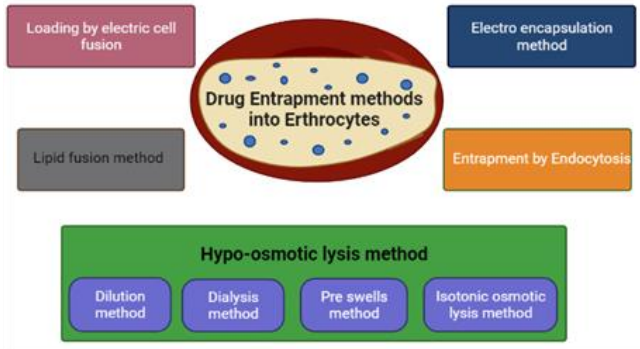


Figure 3: Methods of drug loading or entrapment methods for erythrocytes

A) Hypo-osmotic lysis method

This technique relies on the flexibility of the erythrocytes to bear reversible swelling once placed in an exceedingly hypotonic solution. To accommodate the incoming volume of water, the cell swells up to 1.6 times its original volume, and its shape charges from biconcave to spherical, but the surface area remains unchanged. When the tonicity of the answer exceeds above 150 mOsm/kg, then the erythrocytes burst and form

erythrocyte ghosts. However, just before lysis, transient pores measuring about 200-500 D in diameter appear on the cell wall. These pores facilitate equilibrating the intracellular and extracellular volumes of the erythrocytes. These pores facilitate equilibrating the intracellular and extracellular volumes of the erythrocytes. When this ruptured membrane gets resealed and therefore the cell regains its normal biconcave shape. [9, 10]

The hypo-osmotic method has been further divided into the subsequent four methods:

a) Dilution method
b) Dialysis method
c) Pre swells method
d) Isotonic osmotic lysis method

a) Dilution method:

This was the primary method to be investigated for the entrapment of medicine into the erythrocytes. It's an easy and fast technique.

To a known volume of packed erythrocytes, about 2-20 times the quantity of aqueous drug solution is added. A hypertonic solution is then added to restore the tonicity of the solution. the supernatant is obtained by centrifugation process, this supernatant is discarded, and erythrocytes are washed with an isotonic solution.

Applications for dilution method

This technique is often used for targeting the medicine to the RES organs. Examples embrace enzymes like antineoplastic drugs Examples include enzymes such as asparaginase, β-galactosidase, β-glycosidase, etc., bronchodilators (salbutamol).

b) Dialysis method:

Erythrocyte suspension and drug solution are mixed to get a hematocrit value of 70-80. The mixture is then added to a dialysis tube whose ends are tied by a thread. An air bubble occupying 25 percent of the internal volume is allowed to develop within the tube. The dialysis tube is immersed in 100 ml of swelling solution at 4 °C for the desired lysis time. The tube is periodically agitated using the strings tied at its ends. The dialysis tube is finally immersed in a resealing solution i.e., phosphate buffer saline (pH 7.4) at 25-30 °C. The loaded erythrocytes are washed with the cold resealing solution at 40 C and then suspended in the same solution.

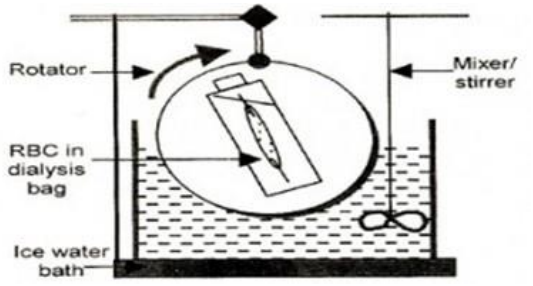


Figure 4: Schematic representation of Erythrocytes dialyzer apparatus for entrapping proteins into erythrocytes using Dialysis method [18].

Applications for Dialysis method:

This can be used for entrapping enzymes like Asparaginase, β-galactosidase, etc., and drugs like gentamicin, Adriamycin, puromycin, etc.

c) Pre swells method:

The erythrocytes are made to undergo controlled swelling by immersing them in the slightly hypotonic solution. The mixture is centrifuged at low speeds to recover the swollen cells. Then relatively small amounts (100-200µL) of the aqueous drug solution are added periodically until the point of lysis. The mixture is centrifuged after each addition of the drug. The point of lysis is detected by the disappearance of the supernatant upon centrifugation. The cells are added to the calculated amount of the hypertonic solution to adjust their tonicity and incubated at 37 °C. [9]

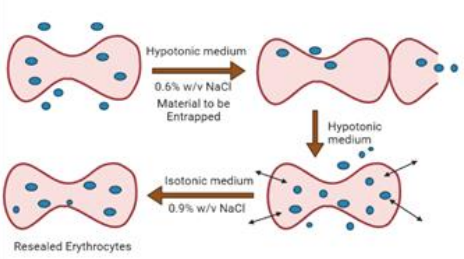


Figure 5: Schematic diagram of Pre swell method

d) Isotonic osmotic lysis method:

This method is also referred as the osmotic pulse method. It is based upon the principle the when the erythrocytes are immersed in the solutions containing the drugs/agents with high membrane permeability, then these solutions diffuse into the cells. Such diffusion is followed by the inflow of water to maintain the osmotic equilibrium the lyses erythrocytes are then incubated in a buffer's isotonic solution at 37°C to enable their resealing. Several drugs/agents used were urea polyethylene glycol, ammonium chloride, etc., [9]

B) Electro-insertion or Electro encapsulation method

An electrical pulse method to encapsulate bioactive molecules was tried by Zimmermann in 1973. Also referred to as electroporation, the tactic is predicated on the observation that electric shock brings about irreversible changes in an erythrocyte membrane. During this technique red blood cell membrane is open by a stuff breakdown; after the pore of erythrocytes is typically resealed by incubation at 37 °C in an isotonic medium.

Applications electro-insertion or electro encapsulation method:

The various chemical encapsulated into the erythrocytes are related and connected 8- amino quinolone, periwinkle plant derivative major tranquilizer, and related thiazine, propranolol, and fat-soluble vitamin.

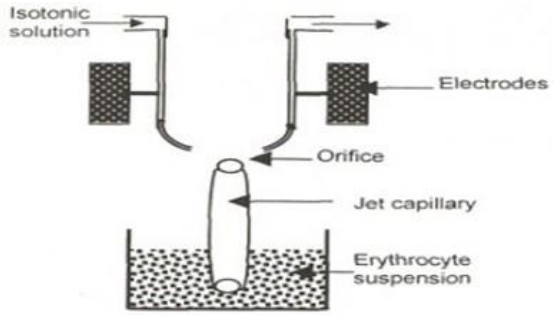


Figure 6: Drug loaded by Electro encapsulation method [40]

C) Entrapment by Endocytosis:

In 1975, Schrier et al., reported this method. This technique involves the addition of one volume of washed-packed erythrocytes to 9 volumes of buffer containing two 5 MM ATP, 2.5 MM mgcl2 and 1MM CaCl2, followed by incubation for two minutes at room temperature. The pores created by this method are resealed by using 154 MM of NaCl and incubate at 37 °C for two minutes. [11, 12, 13, 14]

Applications of endocytosis:

Several chemicals are entrapped in erythrocytes by this technique are antimalarial and related 8- aminoquinoline, a periwinkle plant derivative, major tranquillizer, and related phenothiazines, adrenal cortical steroid, local anesthetic, and vitamin A.

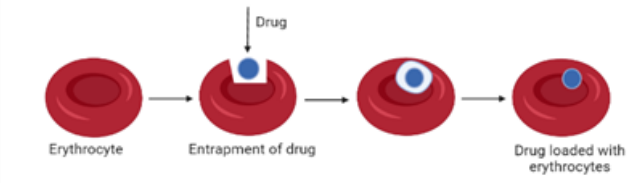


Figure 7: Schematic diagram of drug entrapment by endocytosis

D) Lipid fusion method:

The lipid vesicles containing a drug are often directly fused to human erythrocytes, which cause an exchange with a lipid 161 entrapped drug. The methods are useful for entrapping inositol monophosphate to enhance the oxygen-carrying capacity of cells and the entrapment efficiency of this method is extremely low (~1%). [15]

E) Loading by electric cell fusion:

In this method the initial loading of drug into erythrocytes followed by adhesion of cells to focus on the cells. The fusion is accentuated by the appliance of an electrical pulse, which causes the discharge of an entrapped molecule. An example of this method is loading a cell-specific antibody into an erythrocyte ghost. An antibody against a selected surface protein of target cells is often chemically cross-linked to drug-loaded cells that might direct these cells to desired cells. [16, 17, 18]

3. Release Mechanism of Loaded Drugs

There are primarily 3 ways for a drug release from the Erythrocyte carriers.

a) **Phagocytosis**: By the method of phagocytosis usually RBC removed from the blood circulation. The degree of cross linking determines whether or not liver or spleen can preferentially take away the cells.

b) **Diffusion through the membrane of the cells**: Diffusion of drug molecule through the membrane depends on penetration through a lipid bilayer i.e. bioactive compound have lipid solubility.

c) **Using specific Transport system**: Most of the drugs enter cells by a specific membrane protein system because the carriers are proteins with many properties analogous to that of enzymes.

4. Route of Administration

Intraperitoneal injection reported that survival of cells in circulation was equivalent to the cells administrated by I.V injection. They reported that as 25% of resealed cells 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. SC route for slow release of entrapped agents. They reported that the loaded cell releases encapsulated molecules at the injection site. [19, 20, 21]

5. Storage of Resealed Erythrocytes

One of the major factors which influence the efficiency of resealed erythrocytes as drug carriers is their proper in0vitro storage. [22] This is because the viability and drug content of these resealed erythrocytes can be assured only through proper storage. Several techniques have been devised for proper in-vitro storage of resealed erythrocytes, a few of which have been described below.

1. Usage of Hank's balanced salt solution (HBSS) as a storage media. The cells are suspended in this medium and maintained at 4 ºC. It has been found that such cells are able to retain their physiological characteristic for about 2 weeks.
2. When calcium chelating agents or purine nucleosides have been added to resealed erythrocytes, an enhancement in their circulatory half-life was observed.
3. When the resealed erythrocytes were initially treated with membrane-stabilizing agents (dimethyl sulphoxide, glutaraldehyde etc.,) and then localized or filtered through sintered glass filters, they exhibit enhanced stability upon storage. However, the presence of high levels of membrane-bound stabilizing agents reduces the circulatory half-life of resealed erythrocytes.
4. Entrapping a prodrug that gets converted into the parent drug only at body temperature.

Table 1: Comparison between percent drug loading, advantages as well as disadvantages of different osmosis-based systems

Method	% Drug loading	Advantages	Disadvantages
Dilution	20-40	Fastest and simplest especially for low molecular weight drugs	Entrapment efficiency is less
Dialysis	30-45	Better in-vivo survival of erythrocytes better structural integrity and membrane.	Time consuming, heterogeneous size distribution of resealed erythrocytes.
Pre swell	30-90	Good retention of cytoplasm and good survival in-vivo	----
Isotonic osmotic lysis	-----	Better in-vivo survival	Impermeable only large molecules, process is time consuming.

5. Cryopreservation of erythrocytes at liquid nitrogen temperature.

6. Evaluation of Resealed Erythrocytes

a) Shape and Surface Morphology:

After administration, the morphology of erythrocytes determines their lifetime. In comparison to untreated erythrocytes, the morphological characterization of erythrocytes is conducted using either transmission (TEM) or scanning microscopy (SEM). Certain techniques, like phase contrast microscopy, also can be used. [23]

b) Drug Content:

Cell drug content determines the effectiveness of the tactic utilized in trapping. The process involves deproteinizing sealed, packed cells (0.5 ml) with 2.0 ml acetonitrile undergoes centrifugation process for 10 min at 2500 RPM. Spectrophotometrically, the clear supernatant is analyzed for the drug content. [3]

c) Cell Counting and Cell Recovery:

This includes counting the number of red blood cells per unit volume of whole blood, which is typically calculated by counting the number of intact cells per cubic mm of packed erythrocytes before and after the medication is loaded using an automated system. [11]

d) Erythrocyte Sedimentation Rate:

It is an approximation of RBC's suspension stability in plasma and is related to the number and size of red cells and related plasma protein concentration, especially fibrinogen and α, β globulins. [24]

e) Osmotic shock:

1ml of 10% erythrocyte suspension was diluted with 5 ml of water and centrifuged the above mixture at 3000rpm for 15minutes. The supernatant was estimated for teens' Hb release spectrophotometrically. [20]

f) Turbulence shock:

Turbulence shock is determined by the passage of RBC cell suspension through the needles with a little internal diameter (30 gauges) or vigorously shaking the cell suspension. In each case, hemoglobin and drug are resealed once the procedure is determined. [25, 26]

g) Determination of Entrapped Magnetite:

For determining the concentration of specific metal within the sample, the atomic absorption spectroscopic method is reported. Using atomic absorption spectroscopy, the HCl is added to a hard and fast amount of magnetite bearing erythrocytes, and content is heated at 600C for two hours, then 20% w / v trichloro ethanoic acid is added, and the supernatant is obtained after centrifugation. [23]

h) In-vitro Drug Release and Hemoglobin Content:

The cell suspensions are stored at 40°C in an ambered glass container (5% hematocrit in PBS). Periodically clear supernatants are drawn using a 0.45 filtered hypodermic, deprotected using alcohol, and extensive drug content has been calculable. The supernatant of every sample is often measured using the formula percent Hb release = A540 of 100% Hb history test A540 after centrifugation collected and assayed. [23]

7. Applications of Resealed Erythrocytes

Bioactive agents have targeted to RES damaged erythrocytes are quickly cleared from the circulation by phagocyte Kuffler cells in the liver and spleen. by modifying their membranes, will so be accustomed target the liver and spleen The various approaches to switch the surface characteristics of erythrocytes include surface modification with antibodies, glutaraldehyde, carbohydrates like sialic acid and sulfhydryl. [27]

a) Targeting the liver

Many metabolic disorders associated with deficient or missing enzymes are often treated by injecting these enzymes. However, the problem of exogenous enzymes therapy includes a shorter circulation half- lifetime of enzymes, allergies, and is toxic. [3]

b) Treatment of hepatic tumors

Hepatic tumors are one of the foremost prevalent sorts of cancer. Antineoplastic medications like amethopterin, bleomycin, asparaginase and Adriamycin are with success delivered by erythrocytes. Agents like daunorubicin diffuse rapidly from the cells upon loading and hence pose a drag. This drawback is usually overcome by covalently linking daunorubicin to the erythrocytic membrane using glutaraldehyde or cisaconitic acid as a spacer. The resealed erythrocytes loaded with carboplatin show localization in liver. [27, 28, 29]

c) Treatment of parasitic diseases

The ability of resealed erythrocytes, selectively accumulate within RES organs, which makes them useful for the delivery of anti-parasitic agents. Parasitic diseases that involve harboring parasites within the RES organs are often successfully controlled by this method. Results were favorable in studies involving animal models for erythrocytes loaded with antiprotozoal drugs, anti-leishmanial and anti-amoebic drugs. [3, 25, 30]

Removal of RES iron overload

Deferoxamine-loaded erythrocytes are wont to treat excess iron accumulated due to multiple transfusions to thalassemic patients. Targeting this drug to the RES is extremely beneficial because the aged erythrocytes are destroyed in RES organs, which finish up in an accumulation of iron in these organs. [14]

d) Erythrocytes as Circulating Bioreactors

Erythrocytes are realized as carriers for enzymes to function as circulating bioreactors. Sometimes it's desirable to decrease the extent of circulating metabolites that will enter erythrocytes. It is used as a circulating bioreactor for the controlled delivery system of antiviral drugs.

e) Erythrocytes as Carriers for Enzymes

Enzymes are often injected into the bloodstream to exchange a missing or deficient enzyme in metabolic disorders or to degrade toxic compounds accumulated within the blood thanks to a disease likewise, environmental, lysosomal storage disorders like Gaucher's disease, hyperphenylalaninemia and renal failure are only a few samples of metabolic disorders which will be treated by administration of enzymes. [31]

f) Targeting reticuloendothelial system (RES) organs:

- Surface modification with antibodies
- Surface modification with glutaraldehyde
- Surface modification with carbohydrates like 2-hydroxybenzoic acid
- It's used for Improvement in oxygen delivery to tissues
- It's used for the Microinjection of macromolecule [32]

g) Targeting Non RES:

- Entrapment of magnet particles in conjunction with the drug
- Entrapment of photosensitive material [33]
- Antibody attachment to erythrocyte membrane to urge specificity of action of enzymes [34]

Erythrocytes as Carriers for Drugs:

For the effective treatment of parasitic disease, where the active agents encapsulated in erythrocytes are developed for the slow and sustained release in circulation. Resealed erythrocytes act as a perfect carrier for antineoplastic agents, antimicrobial drugs, vitamins and steroids. [40]

8. Conclusion

The resealed erythrocytes incorporate a specialized result for secure and certain delivery of various medications for passive and active targeting. The concept needs further optimization to become a routine drug delivery system and we can also use the same concept for extended to the delivery of biopharmaceuticals and far remains to be explored relating to the potential of resealed erythrocytes. The preparation of resealed erythrocytes is very simple. These are several techniques identified now a day by which we can easily entrap the drug into erythrocytes. It is having of applications in-vitro as well as in-vivo.

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Conflict of Interest

The author(s) confirm that this article content has no conflict of interest.

Table 2: Examples for Erythrocytes as drug carrier

Erythrocytes as drug carriers	Drug	Method	Reference
Antineoplastic drugs	Actinomycin D	Antineoplastics encapsulated in erythrocytes to direct them selectively to the reticulo-endothelial system involved actinomycin D. using a method of hypotonic exchange-loading reaction.	[35]
	Etoposide	Etoposide is an inhibitor of the topoisomerase revealing a toxic action on tumour cells of macrophage origin. The encapsulation of etoposide in mouse erythrocytes by hypotonic dialysis a greater uptake of etoposide by the macrophages mainly by a process of phagocytises.	[35]
Systemic corticosteroids	Dexamethasone	Loaded erythrocytes containing have been used in vivo in rabbits and humans. Dexamethasone entrapped within rabbit erythrocytes was slowly released from the loaded cells in vivo. The encapsulated drug had a much longer half-life than when free drug was administered intravenously. Patients with chronic obstructive pulmonary disease.	[36]
Angiotensin-converting enzyme inhibitors	Enalaprilat	Enalaprilat is an angiotensin converting enzyme inhibitor, widely used in the treatment of hypertension and congestive heart failure. Human loaded erythrocytes with enalaprilat using a hypotonic pre-swelling method release the drug in vitro according to zero-order kinetics.	[37, 38]
Anti-infective agents	Gentamicin	gentamicin has been studied in vivo encapsulated in human erythrocytes as a selective carrier system to the reticulo-endothelial system and as a potential slow-release carrier	[39]
	Metronidazole	The amino glycoside antibiotic Tests have also been performed involving erythrocytes treated with glutaraldehyde. As slow-release systems with other anti-parasite drugs, such as metronidazole.	[35]

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