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Review Article

Review On Resealed Erythrocytes As A Novel Drug Delivery System

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ABSTRACT

A new strategy that combines inventive development, formulation, new technology, and novel methodology for delivering pharmaceutical compounds in the body as needed to successfully achieve their targeted pharmacological effect is known as a novel drug delivery system. There are numerous novel drug delivery systems available. Among them, "drug- loaded erythrocytes" are one of the most expanding and prospective systems for the delivery of medications and enzymes. Erythrocytes are natural components that make them suitable for use as drug delivery systems to enhance the pharmacokinetics, bioavailability, and many other properties of several medications. Erythrocytes are highly beneficial for targeted and controlled drug delivery systems. Erythrocytes are biocompatible, biodegradable, have a long circulation half-life, and can be loaded with a range of biologically active substances. The aim of the present review is to focus on various features, composition, advantages, disadvantages, isolation, drug loading methods, storage, route of administration, evaluation, application, and novel approaches, future perspectives to resealed erythrocytes.

INTRODUCTION

The pharmacokinetics, biocompatibility, and many other properties of various medications may be enhanced with a good drug delivery system [1]. Some scientists concentrate on transforming blood cells into natural carriers for IV drug delivery [2]. Among these carriers, the erythrocyte-derived delivery system, also known as erythrocyte carriers, attracted the most attention as a biocompatible, biodegradable, and readily available drug carrier [1]. GM. Ihler and colleagues published the first study on utilizing erythrocytes as medication carriers in 1973. In order to treat Gaucher's disease, their team effectively loaded beta-glucosidase and beta-galactosidase into erythrocytes [3]. The main goal of developing this drug delivery system is to maximize therapeutic effectiveness while minimizing side effects and increasing patient compliance [4]. The most numerous cells in the human body, erythrocytes, have the potential to transport medicines [5]. Erythrocytes are unique

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and effective carriers due to their biocompatibility, non-pathogenicity, and biodegradability [6]. They also have zero-order drug release kinetics and a longer half-life [9]. The majority of resealed erythrocytes employed as drug carriers are quickly picked up from the blood by macrophages of the reticuloendothelial system, which are found in the liver, lung, and spleen of the body [4]. Drug-loaded carrier erythrocytes can be made by simply taking blood samples from the target and then resealing the cellular carriers. These carriers are hence known as "resealed erythrocytes" [8].The resealed erythrocytes act as slow-circulating depots upon reinjection, allowing the medicines to be targeted to the reticuloendothelial system and preventing enzymatic destruction [7]. Therapeutic proteins (enzymes and vaccines), nucleic acid, oligosaccharides, cancer chemotherapy, chemical markers, and other active agents like antiviral medication and metabolic modulators are the substances that have been loaded or associated with erythrocytes [10].

Erythrocyte

The most common type of blood cell are red blood cells, commonly known as erythrocytes. RBC is used to deliver oxygen to the body via blood [13]. Erythrocytes are produced in bone marrow, and the process of producing erythrocytes is known as erythropoiesis [12]. In humans, the total number of erythrocytes in males is about 5.4 million cells/mm3 of blood, and in females, it is about 4.8 million cells/mm3 of blood. Erythrocytes are biconcave discs having a diameter of 7.8 micrometers, a thickness of 2.5 micrometers in the periphery, 1 micrometer in the center, and a volume of 85–91 m3 [8]. Chemical constituents of erythrocytes include 63% lipids (0.5%), glucose (0.8%), minerals (0.7%), and non-hemoglobin (33.67%). The life span of an erythrocyte is around 120 days [12].

Isolation Of Erythrocytes:

- Fresh whole blood should be utilized to isolate erythrocytes.
- Fresh whole blood is described as blood that has been drawn, instantly refrigerated to 4°C, and kept in storage for fewer than two days.
- Blood is extracted from a puncture of the heart or spleen in small animals or through veins in mammals and placed in a syringe with an anticoagulant drop.
- The blood is then put into tubes that have been heparinized.
- The collected whole blood is then spun in a chilled centrifuge for 5 minutes at a speed of 2500 rpm and a temperature of 4±1 ºC.
- Carefully remove the buffy coats and serum after centrifugation, and then wash the packed cells at least three times in a phosphate-buffered saline solution with a pH of 7.4.
- Phosphate-buffered saline is then used to dilute the cleansed erythrocytes.
- The washed erythrocytes are then stored in an acid-citrate-dextrose buffer at 4 °C for up to 48 hours before use [14].

S.No	Species	Washing Buffers	Centrifugal Force (g)
1.	Rabbit	10mmol KH2PO4/NaHPO4	500-1000
2.	Dog	15mmol KH2Po4/NaHPO4	500-1000
3.	Human	154mmol NaCl	<500
4.	Mouse	10mmol KH2PO4/NaHPO4	100-500
5.	Cow	10-15mmol KH2PO4/NaHPO4	1000
6.	Horse	2mmol MgCl2, 10mmol glucose	1000
7.	Sheep	10mmol KH2PO4/NaHPO4	500-1000
8.	Pig	10mmol KH2PO4/NaHPO4	500-1000

Table No. 1: The Isolation Of Erythrocyte Involves Various Conditions And Centrifugal Force [11]

Resealed Erythrocytes

These erythrocytes can be made into drug-loaded carriers by simply taking a blood sample from the target organism, isolating the erythrocytes from the plasma, entrapping the medication inside the erythrocytes, and then resealing the cellular carriers that were created. These carriers are hence known as resealed erythrocytes. The reaction of these cells under osmotic conditions serves as the foundation for the entire process. The drug-loaded erythrocytes act as slow-circulating depots after reinjection and direct the medication to the reticuloendothelial system (RES) [13].

Advantages:

- Biocompatible, especially when using autologous cells, preventing the chance of an immune reaction being triggered.
- Biodegradability with no hazardous product formation.
- The carrier's size and shape are remarkably consistent.
- A tiny volume of cells can enclose a relatively inert intracellular environment.
- Large amounts of substances can be loaded, and isolation is simple.
- Preventing endogenous chemical inactivation from causing the loaded medication to degrade.
- A wide range of chemical entrapments are conceivable.
- Drug entrapment is feasible without subjecting the substance to be entrapped to chemical change.
- It is possible to keep plasma concentrations in a constant state and reduce concentration volatility.
- Protection of the body against medication toxicity.
- Concentrating on the RES's organ.
- The ideal drug release kinetics are zero-order.
- Extend the duration of the drug's systemic activity in the body.
- Achieving steady-state plasma concentration with the potential for zero-order drug release kinetics.
- Changing the drug's pharmacokinetic and pharmacodynamic characteristics.
- There is a considerable reduction in negative effects.
- Large amounts of medication that can fit inside a tiny volume of cells guarantee dose sufficiency.
- The capacity to target RES organs [10,15,16,17].

Disadvantages:

- Their ability to function as carriers of nonphagocyte target tissues is restricted.
- There may be a chance for cell clumping and dosage dumping.
- The loaded erythrocytes rapidly leak some substance that has been enclosed.
- A number of substances could change the physiology of erythrocytes.
- It is not possible to inject directly into the cell nucleus.
- A two-week storage shelf life.
- Economical method [5,13].

Requirements For Encapsulation

- Erythrocytes can entrap a variety of biologically active substances (5000–60,000 dalton).
- In salts, erythrocytes can entrap non-polar molecules. For example, bovine RBC can significantly entrap tetracycline HCl salt.
- In general, molecules should be both polar and nonpolar when they are trapped.
- By absorbing other molecules, hydrophobic substances can become stuck in erythrocytes.
- Charged molecules are kept longer after being enclosed than uncharged molecules. When the molecule is smaller than sucrose and larger

than B-galactosidase, the size of the molecule entrapped plays an important role [13,15].

- Factors That Take Resealed Erythrocytes Into Consideration As A Carrier:
- Its size and form allow it to flow through capillaries.
- The unique physico-chemical characteristics that can be used to identify a required site.
- Its biocompatibility and low toxic nature.
- After the medicine has been released at the target site, its breakdown product should be biocompatible.
- There should be minimal medication leakage before the target site is reached.
- Its well-managed medication release pattern.
- High drug loading efficiency for a wide range of medications with various qualities.
- The drug's physical-chemical compatibility [15].

There Are Two Ways That Erythrocytes Can Be Exploited As Carriers:

- Targeting a certain tissue or organ: Only the erythrocyte membrane is used for targeting. This is carried out by dividing the cell in a hypotonic solution, adding the medication, and allowing the split cells to reseal into spheres. Red-cell ghosts are the name given to such erythrocytes.
- Erythrocytes can also be used as a continuous or prolonged release system for drugs, which prolongs the duration of the drug's action. There are various ways to encapsulate medications into erythrocytes. They circulate for extended periods of time (up to 120 days) and slowly and steadily release the medicine they contain [10,18].

Drug loading Technique For Resealed Erythrocytes :

a. Hypo-osmotic lysis
b. Dilution method
c. Preswelling method
d. Dialysis method
e. Osmotic lysis method
f. Membrane perturbation method
g. Electro encapsulation method
h. Endocytosis method
i. Lipid fusion method
j. Electric cell fusion method
k. Use of Red cell loader

Hypo-osmotic lysis method:

By means of osmotic lysis and resealing, the intracellular and extracellular solutes of erythrocytes change. The medicine must be enclosed by the red blood cell membrane using this technique [19].

Dilutional technique or hypotonic dilution:

One of the most straightforward and quick methods for encapsulating compounds into erythrocytes is hypotonic dilution. In this procedure, 2–20 liters of a drug's aqueous solution are added to packed erythrocytes to dilute them. The solution's tonicity is then restored by the addition of a hypertonic buffer. Following the removal of the supernatant liquid from the prepared combination, the pellet is washed with an isotonic buffer solution. Low entrapment efficiency and a large loss of hemoglobin and other cell components are the method's main drawbacks. Which ultimately shortens the laden cells' half-life in circulation. These cells are easily phagocytized by RES macrophages; hence, they are employed for targeting RES organs. The loading of bronchodilators like salmeterol and enzymes like B-galactosidase and B-glucosidase, asparaginase, and arginase is typically done using the hypotonic dilution approach [20].

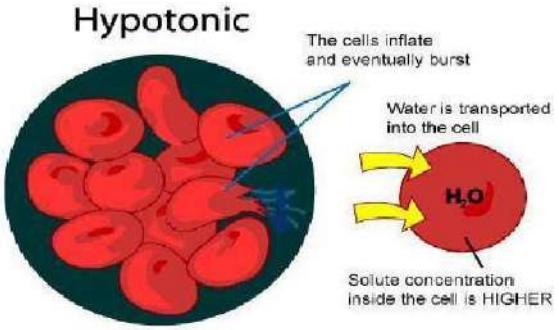

Fig. No.1: Hypotonic dilution.

Hypotonic preswelling method:

This approach is based on the idea that erythrocytes are first swollen without being lysed by being kept in a hypotonic solution. This technique was created by Rechsteiner in 1975. By centrifuging at a low speed, the enlarged cells are given the chance to recover. At the point of lysis, relatively little aqueous drug solution is introduced. The benefits of this approach are that it is quick, easy, and just slightly harms cells. The detection point is taken into consideration when the barrier between the cell lines and the supernatant disappears because of gravitational force. It is possible to restore the tonicity of a cell mixture to its lysis point by adding an appropriate amount of hypertonic buffer. The cell suspension is incubated at 37 °C to seal the erythrocytes once more. Similar to regular cells, these cells have an extended circulation half-life. Propranolol, levothyroxine, metronidazole, isoniazid, cortisol-21phosphate, prednisolone-21-sodium, cyclophosphamide, -1 antitrypsin, interferon alpha-2, and insulin are among the medications that can be encased in erythrocytes using this technique [10].

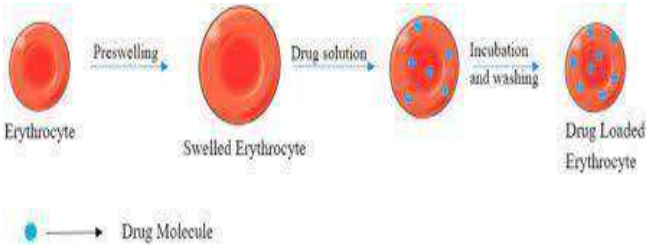

Fig. No. 2: Hypotonic Preswelling Method.

Hypotonic dialysis method:

This technique for loading enzymes and lipids was first described in 1959 by Klibansky, used in 1977 by Deloach and Ihler, and used in 1989 by Gaudreault RC. Mixing cleansed erythrocyte suspension with a medication solution containing phosphate buffer (pH 7.4) results in the desired hematocrit. The dialysis bag is filled with this mixture, and the bag's ends are sealed with thread. There is still an air bubble inside the tube which is around 25% of its internal capacity. A bubble is used during dialysis to mix the substances. After that, the dialysis tube is sealed in 200 ml of isotonic PBS pH 7.4 at a temperature of 25 to 30 0C. The therefore acquired loaded erythrocytes are subsequently washed with cold PBC at 40 0C. The cells are finally resuspended in PBC. Gentamicin, adriamycine, erythropoietin, furamycin A, IgG, and other examples of encapsulated drugs [11].

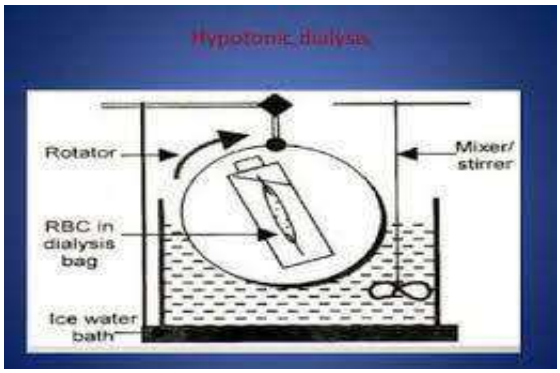

Fig. No. 3: Hypotonic Dialysis Method.

Isotonic osmotic lysis method:

This technique, also known as the osmotic pulse technique, uses chemical or physical processes to cause isotonic hemolysis. It's possible that the isotonic solutions are not isotonic. The solute will diffuse into the red blood cells as a result of the concentration gradient if the red blood cells are incubated in solutions with high membrane permeability. This is followed by an input of water to maintain the osmotic balance. For the isotonic hemolysis procedure, substances like polyethylene glycol and ammonium chloride have been utilized. However, this strategy is equally susceptible to

modifications in the make-up of the membrane structure. A method for suspending RBC in an isotonic solution of dimethyl sulfoxide (DMSO) was developed by Franco et al. in 1987. The solution was diluted with an isotonic buffered medication solution. After being divided, the cells were once again shut [14].

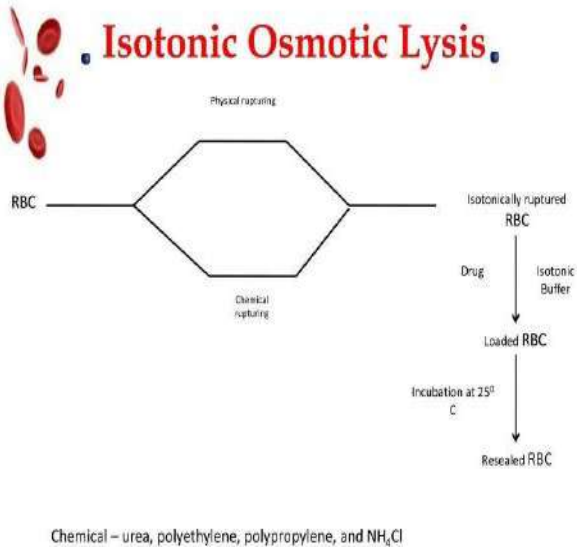

Fig. No. 4: Isotonic Osmotic Lysis Method.

Membrane chemical perturbation method:

This technique is based on the fact that when erythrocytes are exposed to specific substances, their membrane permeability increases. In 1973, Deuticke demonstrated that exposure to a polyene antibiotic like amphotericin B enhances the permeability of the erythrocytic membrane. In 1980, Kitao and Hattori successfully applied the technique to entrap the anticancer medication daunomycin in human and animal erythrocytes. Halothane was utilized for the same function by Lin et al. These techniques, however, cause the cell membrane to undergo irreversible, damaging alterations, which makes them unpopular.

Several encapsulating agents are: daunomycin [21].

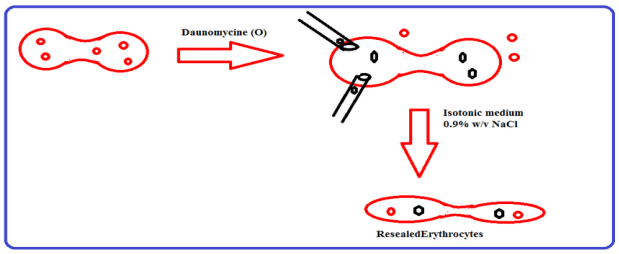

Fig. No. 5: Membrane Chemical Perturbation

Electro-encapsulation method:

The approach treats electrically induced permeability changes at high membrane potential differences. By applying a variable voltage of 2 kV/cm for 20 seconds and membrane polarization for microseconds, an electrical breakdown is produced. In either case, depending on the size of the pores, ions readily distribute between the extracellular and intracellular spaces to reach equilibrium. The potential difference across the membrane is created either directly by inter- and intracellular electrodes or indirectly by applying an internal electric field to the cells once the membrane has been penetrated. Despite its cytoplasmic macromolecules, the membrane is still impenetrable. The colloidal osmotic pressure of hemoglobin in red blood cells is around 30 mOsm. This pressure causes an influx of water and ions. When the cell volume exceeds 155% of its initial value, the membrane is ruptured. The rationale for preventing a breakdown is to balance the colloidal osmotic pressure of cellular macromolecules, since colloidal osmotic swelling is what causes cell breakdown. By including macromolecules, ribonucleases, and proteins like bovine serum albumin, this can be changed. Pores remain open at 4 oC for a few days under this osmotically regulated circumstance. Drug molecules will enter erythrocytes if they are added at this location. Primaquine and related 8-aminoquinolines, vinblastine, chlorpromazine and related phenothiazines, hydrocortisone, propranolol, tetracaine, and vitamin A are among the several possibilities captured by this technique [21].

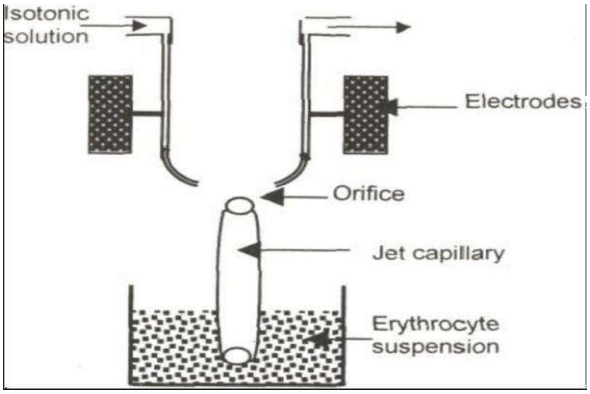

Fig. No.6: Elecroencapsulation method

Endocytosis method:

Schrier (1987) reported on this technique. One volume of washed-packed erythrocytes is added to nine volumes of buffer containing 2.5 mm ATP, 2.5 mm MgCl2, and 1 mm CaCl2, and then the mixture is incubated for 2 min at room temperature to initiate endocytosis. Re- sealing the holes produced by this procedure requires 154 mm of Nacl and two minutes of incubation at 370 °C. Endocytosis causes the substance to become entrapped. Endocytosed material is protected from erythrocytes by being separated from the cytoplasm by the vesicle membrane, and vice versa. Examples of agents in encapsulation: Vitamin A, vinblastine, chlorpromazine, hydrocortisone, propranolol, vitamin A, primaquine, vinblastine, etc [22].

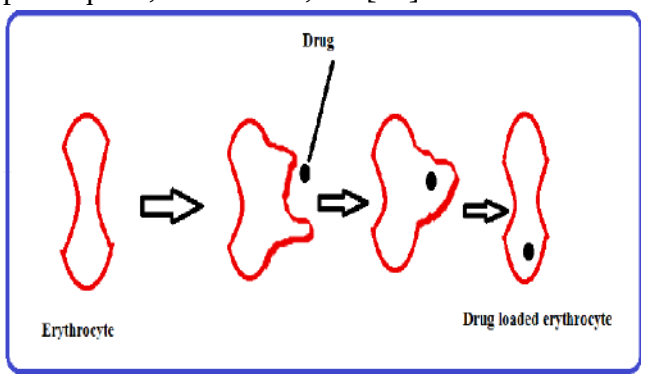

Fig. No.7 :Endocytosis Method.

Lipid fusion method:

This technique involves fusing human erythrocytes with lipid vesicles that contain bioactive molecules, which results in the exchange of drug molecules that are trapped in lipid. This approach offers relatively poor encapsulation effectiveness. Inositol-hexaphosphate- containing lipid vesicles and human erythrocytes were united by Nicola and Gresonde. The oxygen affinity for hemoglobin in intact erythrocytes was significantly reduced by the incorporation of inositol hexaphosphate. Tyrosine kinase was reportedly sealed back into human erythrocytes by Harrison et al. via fast freezing and thawing in liquid.

Several encapsulating agents include: Monophosphate inositol [11].

Loading by electric cell fusion:

In this technique, drug molecules are first loaded into erythrocyte ghosts, and then these cells adhere to the target cells. An electric pulse is used to accelerate the fusion, which results in the release of a trapped molecule. The incorporation of a cell-specific monoclonal antibody into an erythrocyte ghost is a good example of this technique. Chemical crosslinking can drive drug-loaded cells to target cells by binding an antibody to a particular surface protein on the target cell [11].

Use of a red cell loader:

A unique technique for the trapping of non-diffusible medicines within human erythrocytes was created by Magnani and colleagues in 1998. The "red cell loader" was the name given to the apparatus created for this technique. The technique only needs 50 ml of blood sample, and it entraps several biologically active substances into erythrocytes over the course of two hours at room temperature under blood banking conditions. The procedure involves concentration using a hem filter, two consecutive hypotonic dilutions of washed erythrocytes, isotonic resealing of the cells, and two hypotonic dilutions of the cells. 30% of the drug was loaded, and 35–50% of the cells recovered. The treated erythrocytes survived in vivo normally. By making the same cells appear closer to tissue macrophages, the same cells might be used for targeting [11].

Fig. No.8: Use of Red cell Loader

In Vitro Storage:

Resealed erythrocytes' ability to distribute drugs successfully is more heavily influenced by how they are stored in vitro. The most popular storage options include acid-citrate-dextrose at 40 °C and Hank's balanced salt solution. At this temperature, cells can survive for at least two weeks while retaining their physiologic and carrier features. The inclusion of purine nucleosides or calcium-chelating compounds extends the duration that cells survive after being injected [8].

In Vivo Lifespan:

Reinjected sealed erythrocytes' survival period in the bloodstream after reinjection is the major factor in determining how effective they are. A longer life is needed to achieve sustained action. The size, shape, surface electrical charge, amount of hemoglobin and other cell components lost during loading, and life span of resealed erythrocytes depend on it. Labeling cells with 51Cr, fluorescent markers like fluorescin isothiocyanate, or trapping 14C sucrose or gentamicin are a few of the techniques used to calculate in vivo survival time [8].

Route Of Administration:

According to intraperitoneal injection, the survival of cells in circulation was on the same level as that of cells given through injection. They suggested this type of injection as a strategy for extravascular targeting of RBCs to peritoneal macrophages, reporting that 25% of resealed cells stayed in circulation for 14 days. Subcutaneous channel for delayed release of substances that have been trapped. They reported that encapsulated compounds were released at the injection site by the loaded cell [16].

Evaluation Of Resealed Erythrocytes :

- **Shape and surface morphology:**

By comparing these ghost erythrocytes to untreated erythrocytes using either transmission (TEM) or scanning (SEM) electron microscopy, the morphological examination of these ghost erythrocytes is accepted. When exposed to fluids with varying osmolalities using osmosis-based encapsulation techniques, erythrocytes undergo morphological changes that can be observed using electron microscopy. The life of administered erythrocytes is determined by their morphology [20,23].

- **Drug content:**

The effectiveness of the approach employed to entrap the drug depends on how much of it is present in the cells. The procedure involves deproteinizing packed, loaded cells (0.5 mL) with mL of acetonitrile and centrifuging the mixture for 10 minutes at 2500 rpm. The drug content is spectrophotometrically analyzed in the clear supernatant [20,23].

- **Percent cell recovery and cell counting:**

The number of RBCs per unit volume of whole blood is counted using this method, usually using automated counting. Based on variations in hematocrit and the significance of the erythrocyte suspension both before and after loading, red cell recovery can be determined. To maximize cell recovery, it is important to minimize loss during the encapsulation process
[20,23].

- **Turbulence fragility:**

The fragility of a cell suspension is determined by passing it through needles with a smaller internal diameter (for example, 30 gauges) or by shaking the suspension vigorously. Hemoglobin and

medication discharged during the surgery are identified in both situations. It has been discovered that resealed cells have a higher turbulent fragility [8,10].

- **Drug release:**

Drug loading may result in persistent drug release that affects the loaded erythrocytes' in vivo pharmacokinetic behavior. Using autologous plasma or an isosmotic buffer at 370°C with a hemocrit regulated between 0.5% and 50%, drug leakage from loaded erythrocytes is studied in vitro. At certain times, the supernatant is withdrawn and replenished with an equal volume of autologous plasma or buffer. Some writers suggested utilizing a dialysis bag to conduct in vitro release investigations from loaded erythrocytes [8,10].

- **Erythrocyte sedimentation rate**:

It is a measurement of how stable red blood cells are in suspension in plasma and is based on the quantity and size of the red blood cells as well as the relative concentration of plasma proteins, particularly fibrinogen and globulins. In order to perform this test, blood cells are deposited in a standard tube, and the rate of sedimentation is measured. Blood ESR should range from 0 to 15 mm/hr; a greater rate suggests disease processes that are active but difficult to diagnose [3,24-28].

- **Osmatic fragility:**

The possibility of alterations in the integrity of the cell membrane and the resistance of these cells to the osmotic pressure of the suspension medium are both indicated by the osmotic fragility of resealed erythrocytes. This test is conducted by incubating samples sequentially with isotonic to hypotonic saline solutions and calculating the amount of drug and Hb. Because of increased intracellular osmotic pressure, resealed cells typically have higher osmotic fragility than normal cells [20,23].

- **Determination of entrapped magnetite:**

The atomic absorption spectroscopic method is provided for determining the concentration of a certain metal in the sample. A fixed amount of erythrocytes containing magnetite are treated with HCl, heated at 600°C for two hours, and then treated with 20% w/v trichloroacetic acid. The supernatant obtained after centrifugation is then utilized to measure the magnetite concentration using atomic absorption spectroscopy [8,10].

- **Hemoglobin release:**

Changes in the permeability of the red blood cell membrane during the encapsulation process may result in a reduction in the amount of hemoglobin present in erythrocytes. In addition, there is a connection between the rate of hemoglobin and the rate of medication release from the erythrocytes. Using a red cell suspension and a spectrophotometer, the absorbance of the supernatant at 540 nm is used to measure hemoglobin leakage [8,10].

- **In vitro stability:**

The stability of the loaded erythrocytes is evaluated by incubating the cells in autologous plasma or in an isoosmotic buffer at temperatures between 40 oC and 370 oC, with the hemocrit adjusted between 0.5% and 5% [8,10].

- **In vitro drug release and Hb content :**

Periodically, the in vitro release of drug(s) and hemoglobin from drug-loaded cells is seen. In an amber-colored glass container, the cell suspension (5% hematocrit in PBS) is kept at 4 °C for storage. It is occasionally necessary to remove the supernatant with a hypodermic syringe, deproteinize it with methanol, and filter it through a 0.45-m filter. The presence of drugs or hemoglobin is then estimated. Mean corpuscular hemoglobin is another metric to assess the disposition of hemoglobin upon resealing. It is an index independent of the red cell and measures the average concentration of hemoglobin per 100 ml of cells. As a result, it accurately represents their Hb content [20,35].

- **Miscellaneous :**

Resealed erythrocytes can also be identified by their cell sizes, mean cell volumes, energy consumption, lipid compositions, fluidity of their membranes, rheological characteristics, and ability to separate density gradients [8,10].

Application Of Resealed Erythrocytes :

There are numerous potential uses for resealed erythrocytes in both human and veterinary medicine.

- **Slow release of drugs:**

The continuous delivery of antineoplastics, parasiticides, veterinary antiamoebics, vitamins, hormones, antibiotics, and cardiovascular medicines has been done by using erythrocytes as circulating depots [8].

- **Treatment of hepatic tumors:**

One of the most common types of cancer is hepatic tumors. Erythrocytes have been used to deliver anti-cancer medications such as methotrexate, bleomycin, asparginase, and adriamycin effectively. Agents like daunorubicin, which diffuses quickly from the cells after loading, are problematic [8].

- **Treatment of parasitic diseases:**

Resealed erythrocytes are a helpful tool when delivering antiparasitic drugs since they can specifically collect inside RES organs. This approach can be used to successfully manage parasitic illnesses that involve parasites being harbored in the RES organs [8].

- **Removal of RES iron overload:**

Erythrocytes that have been loaded with desferrioxamine have been used to treat excess iron that has built up as a result of numerous transfusions to thalassemic patients. It is particularly advantageous to target this medication at the RES since these organs accumulate iron because elderly erythrocytes are destroyed there [8].

- **Removal of toxic agents:**

Murine-carrying erythrocytes containing sodium thiosulfate and bovine rhodanese are used to prevent cyanide poisoning. It has also been observed that resealed erythrocytes carrying a recombinant phosphodiesterase can inhibit organophosphorus poisoning [29].

- **Targeting organs other than those of RES:**

Organs outside the RES have recently been the target of resealed erythrocyte therapy. The different strategies include drug and paramagnetic particle entrapment, photosensitive material entrapment, ultrasound wave application, and antibody attachment to the erythrocyte membrane for action specificity [8].

- **Delivery of antiviral agents:**

Antiviral drugs have been entrapped in resealed erythrocytes for effective distribution and targeting, according to a number of papers published in the literature. Since the majority of antiviral medications are nucleotides or nucleoside analogs, consideration must be given to how they enter and exit the membrane. Because nucleosides traverse the membrane quickly but nucleotides do not, they have protracted release profiles. These moieties must be changed into purine or pyrimidine bases in order for nucleotides to be released. Deoxycytidine derivatives, recombinant HSV-1 glycoprotein B, azidothymidine derivatives, and azathioprene have all been delivered using resealed erythrocytes [8].

- **Enzyme therapy:**

Enzymes are frequently employed in clinical practice as medications, replacement therapies for diseases related to their deficiency (such as Gaucher's disease and galactosuria), for the breakdown of hazardous chemicals subsequent to poisoning (such as cyanide and organophosphorus), and for the treatment of diseases associated with their deficiency [8].

- **Improvement in oxygen delivery to tissues:**

The protein hemoglobin is responsible for erythrocytes' ability to transport oxygen. In the lungs, 95% of hemoglobin is saturated with oxygen under normal circumstances, but only 25% of oxygenated hemoglobin deoxygenates in peripheral circulation under physiological settings. As a result, venous blood returns to the lungs while carrying the majority of the oxygen that has been bonded to hemoglobin. It has been suggested that this bound fraction be used to treat a lack of oxygen [8].

- **Microinjection of macromolecules:**

For a variety of cell biological applications, the biological properties of macromolecules, including DNA, RNA, and proteins, are taken advantage of. Thus, these macromolecules are captured in grown cells using a variety of techniques, such as microinjection. Erythrocytes are suitable for the trapping of macromolecules due to their comparatively basic structure and absence of complicated cellular elements (such as the nucleus) [8].

Erythrocyte can also used be used in cancer:

The term "cancer" refers to a group of disorders in which other tissues can be invaded by abnormal cells that divide uncontrollably [8].

Novel Approaches :

- **Erythrosomes:**

These are specialized vesicular structures coated with a lipid bilayer and chemically cross- linked to the surface of human erythrocytes. By adapting a reverse-phase evaporation method, this process is performed. It has been suggested that these vesicles can be effective encapsulation devices for macromolecular medicines [30-32].

- **Nanoerythrosomes:**

These are made by forcing erythrocyte ghosts out of the erythrocyte, resulting in tiny vesicles with a typical diameter of 100 nm. Using gluteraldehyde as a spacer, daunorubicin was covalently coupled to nanoerythrosomes. Both in vitro and in vivo, this combination proved more effective than free daunorubicin alone [33,34].

- **Future Perspectives:**

There is still more useful work to be done to optimize the idea of using erythrocytes as drug or bioactive carriers and to fully utilize their potential for both passive and active medication targeting. Cancer and other diseases would undoubtedly be cured. The existing cellular drug idea can be given a new dimension by combining genetic engineering elements [8]

CONCLUSION

The utilization of resealed erythrocytes as a medication carrier, an enzyme replacement therapy, etc. has received a lot of attention over the past ten years. For the secure and reliable administration of diverse medications for passive and active targeting, the use of resealed erythrocytes appears promising. To become a standard drug delivery method, the idea needs to be improved. The same idea can be applied to biopharmaceutical delivery, and there is still much to learn about the potential of resealed erythrocytes. Erythrocyte carriers are currently thought to be "golden eggs in novel drug delivery systems," given their enormous potential. The majority of research in this field is at the in vitro stage, and there are still active projects globally that need to advance into preclinical and lastly, clinical research to demonstrate the efficacy of this potential delivery technology. In addition, there is an extremely efficient and secure method of administering anti-cancer medication with minimal or no toxicity.

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