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Review Article

RESEALED ERYTHROCYTES: A NOVEL DRUG DELIVERY SYSTEM

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ABSTRACT

Carrier erythrocytes have been evaluated in thousands of drug administration in humans proving safety and efficacy of the treatments. Carrier erythrocytes, resealed erythrocytes loaded by a drug or other therapeutic agents, have been exploited extensively in recent years for both temporally and spatially controlled delivery of a wide variety of drugs and other bioactive agents owing to their remarkable degree of biocompatibility, biodegradability and a series of other potential advantages. Biopharmaceuticals, therapeutically significant peptides and proteins, nucleic acid-based biologicals, antigens and vaccines, are among the recently focused pharmaceuticals for being delivered using carrier erythrocytes. In this review article, the potential applications of erythrocytes in drug delivery have been reviewed with a particular stress on the studies and laboratory experiences on successful erythrocyte loading and characterization of the different classes of biopharmaceuticals.

Key words: Resealed erythrocytes, Drug targeting, characterization methods and Applications.

INTRODUCTION

Erythrocytes, the most abundant cells in the human body, have potential carrier capabilities for the delivery of drugs. Erythrocytes are biocompatible, biodegradable, possess very long circulation half lives and can be loaded with a variety of chemically and biologically active compounds using various chemical and physical methods. Application of erythrocytes as promising slow drug release or site-targeted delivery systems for a variety of bioactive agents from different fields of therapy has gained a remarkable degree of interest in recent years. Biopharmaceuticals are among the most widely exploited candidates for being delivered to the host body using these cellular carriers. In this review, the potential applications of erythrocytes in drug delivery have been highlighted [1-3].

ISOLATION OF ERYTHROCYTES

Various types of mammalian erythrocytes have been used for drug delivery, including erythrocytes of mice, cattle, pigs, dogs, sheep, goats, monkeys, chicken, rats, and rabbits. To isolate erythrocytes, blood is collected in heparinized tubes by venipuncture. Fresh whole blood is typically used for loading purposes because the encapsulation efficiency of the erythrocytes isolated from fresh blood is higher than that of the aged blood [4]. To isolate erythrocytes, blood is collected in heparinized tubes by venipuncture. Fresh whole blood is typically used for loading purposes because the encapsulation efficiency of the erythrocytes isolated from fresh blood is higher than that of the aged blood. Fresh whole blood is the blood that is collected and immediately chilled to 4^0c and stored for less than two days. The erythrocytes are then harvested and washed by centrifugation. The washed cells are suspended in buffer solutions at various hematocrit values as desired and are often stored in acid–citrate–dextrose buffer at 4^0 c as long as 48 h before use. Jain and Vyas have described a well-established protocol for the isolation of erythrocytes. The loading of drugs in erythrocytes was reported separately by Ihler et al. and Zimmermann. In 1979, the term carrier erythrocytes were coined to describe drug-loaded erythrocytes. [5] [6]

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Advantages and Disadvantages of Erythrocytes in Drug Delivery

Advantages [6-15]

1. A remarkable degree of biocompatibility, particularly when the autologous cells are used for drug loading.
2. Complete biodegradability and the lack of toxic product(s) resulting from the carrier biodegradation.
3. Avoidance of any undesired immune responses against the encapsulated drug.
4. Considerable protection of the organism against the toxic effects of the encapsulated drug, e.g. antineoplasms.
5. Remarkably longer life-span of the carrier erythrocytes in circulation in comparison to the synthetic carriers. In the optimum condition of the loading procedure, the life-span of the resulting carrier cells may be comparable to that of the normal erythrocytes.
6. An easily controllable life-span within a wide range from minutes to months.
7. Desirable size range and the considerably uniform size and shape.
8. Protection of the loaded compound from inactivation by the endogenous factors.
9. Possibility of targeted drug delivery to the RES organs.
10. Relatively inert intracellular environment.
11. Availability of knowledge, techniques, and facilities for handling, transfusion, and working with erythrocytes.
12. Possibility of ideal zero-order kinetics of drug release.
13. Wide variety of compounds with the capability of being entrapped within the erythrocytes.
14. Possibility of loading a relatively high amount of drug in a small volume of erythrocytes, which, in turn, assures the dose sufficiency in clinical as well as animal studies using a limited volume of erythrocyte samples.
15. Modification of the pharmacokinetic and pharmacodynamic parameters of the drug.
16. Remarkable decrease in concentration fluctuations in steady state in comparison to the conventional methods of drug administration, which is a common advantage for most of the novel drug delivery systems.
17. Considerable increase in drug dosing intervals with drug concentration in the safe and effective level for a relatively long time.
18. Possibility of decreasing drug side effects.

Disadvantages [16-20]

1. The major problem encountered in the use of biodegradable materials or natural cells as drug carriers is that they are removed in vivo by the RES as result of modification that occurred during loading procedure in cells. This, although expands the capability to drug targeting to RES, seriously limits their life-span as long-circulating drug carriers in circulation and, in some cases, may pose toxicological problems.
2. The rapid leakage of certain encapsulated substances from the loaded erythrocytes.
3. Several molecules may alter the physiology of the erythrocyte.
4. Given that they are carriers of biological origin, encapsulated erythrocytes may present some inherent variations in their loading and characteristics compared to other carrier systems.
5. The storage of the loaded erythrocytes is a further problem provided that there are viable cells and need to survive in circulation for a long time upon re-entry to the host body. Conditioning carrier cells in isotonic buffers containing all essential nutrients, as well as in low temperatures, the addition of nucleosides or chelators, lyophilization with glycerol or gel immobilization have all been exploited to overcome this problem.
6. Possible contamination due to the origin of the blood, the equipment used and the loading environment. Rigorous controls are required accordingly for the collection and handling of the erythrocytes.

METHODS OF DRUG LOADING

Several methods can be used to load drugs or other bioactive compounds in erythrocytes, including physical (e.g., electrical pulse method) osmosis-based systems, and chemical methods (e.g., chemical perturbation of the erythrocytes membrane). The following are types of drug loading: Hypotonic hemolysis, hypotonic dilution, hypotonic pre swelling, isotonic osmotic lysis, Chemical perturbation of the membrane. Electro-insertion or electron capsulation, Entrapment by endocytosis, loading by electric cell fusion, loading by lipid fusion [21].

Hypotonic Hemolysis

This method is based on the ability of erythrocytes to undergo reversible swelling in a hypotonic solution. Erythrocytes have an exceptional capability for reversible shape changes with or without accompanying volume change and for reversible deformation under stress. An increase in volume leads to an initial change in the shape from biconcave to spherical. This change is attributable to the absence of superfluous membrane; hence, the surface area of the cell is fixed. The cells assume a spherical shape to accommodate additional volume while keeping the surface area constant. The volume gain is 25–50%. The cells can maintain their integrity up to a tonicity of 150 mosm/kg, above which the membrane ruptures, releasing the cellular contents. At this point (just before cell lysis), some transient pores of 200–500 Å are generated on the membrane. After cell lysis, cellular contents are depleted. The remnant is called an erythrocyte ghost [9-12].

Hypotonic Dilution

Hypotonic dilution was the first method investigated for the encapsulation of chemicals into erythrocytes and is the simplest and fastest. In this method, a volume of packed erythrocytes is diluted with 2–20 volumes of aqueous solution of a drug. The solution

tonicity is then restored by adding a hypertonic buffer. The resultant mixture is then centrifuged, the supernatant is discarded, and the pellet is washed with isotonic buffer solution. [23] The major drawbacks of this method include low entrapment efficiency and a considerable loss of hemoglobin and other cell components. This reduces the circulation half life of the loaded cells. These cells are readily phagocytosed by RES macrophages and hence can be used for targeting RES organs. Hypotonic dilution is used for loading enzymes such as galactosidase and glucosidase, asparginase and arginase, as well as bronchodilators such as salbutamol. [24-26]

Hypotonic Preswell Technique

This method was investigated by Rechsteiner [26] in 1975 and was modified by Jenner et al for drug loading. This method based on the principle of first swelling the erythrocytes without lysis by placing them in slightly hypotonic solution. The swollen cells are recovered by centrifugation at low speed. Then, relatively small volumes of aqueous drug solution are added to the point of lysis. The slow swelling of cells results in good retention of the cytoplasmic constituents and hence good survival in vivo. This method is simpler and faster than other methods, causing minimum damage to cells. Drugs encapsulated in erythrocytes using this method include propranolol [27], asparginase [28], cyclopohphamide, cortisol-21-phosphate [29, 30], 1-antitrypsin [30], methotrexate, insulin [30, 31], metronidazole [32], levothyroxine [33], enalaprilat [34], and isoniazid [35].

Isotonic Osmoticlysis

This method, also known as the osmotic pulse method, involves isotonic hemolysis. Erythrocytes are incubated in solutions of a substance with high membrane permeability; the solute will diffuse into the cells because of the concentration gradient. Chemicals such as urea solution [36], polyethylene glycol [37], and ammonium chloride have been used for isotonic hemolysis. In 1987, Franco et al. developed a method that involved suspending erythrocytes in an isotonic solution of dimethyl sulfoxide (DMSO) [31].

Hypotonic Dialysis

This method was first reported by Klibansky [20] for loading enzymes and lipids. Several methods are based on the principle that semipermeable dialysis membrane maximizes the intracellular: extracellular volume ratio for macromolecules during lysis and resealing. In the process, an isotonic, buffered suspension of erythrocytes with a hematocrit value of 70–80 is prepared and placed in a conventional dialysis tube immersed in 10–20 volumes of a hypotonic buffer. The medium is agitated slowly for 2 h. The tonicity of the dialysis tube is restored by directly adding a calculated amount of a hypertonic buffer to the surrounding medium or by replacing the surrounding medium by isotonic buffer. The drug to be loaded can be added by either dissolving the drug in isotonic cell suspending buffer inside a dialysis bag at the beginning of the experiment or by adding the drug to a dialysis bag after the stirring is complete [21-25].

Chemical Perturbation of the Membrane

This method is based on the increase in membrane permeability of erythrocytes when the cells are exposed to certain chemicals. In 1973, Deuticke et al. showed that the permeability of erythrocytic membrane increases upon exposure to polyene antibiotic such as amphotericin B. In 1980, this method was used successfully by Kitao and Hattori to entrap the antineoplastic drug daunomycin in human and mouse erythrocytes. Lin et al. used halothane for the same purpose. However, these methods induce irreversible destructive changes in the cell membrane and hence are not very popular. [27-29]

Electro-Insertion or Electro Encapsulation

This method is also known as electroporation, the method consist of creating electrically induced permeability changes at high membrane potential differences. In 1977, T song and Kinosita suggested the use of transient electrolysis to generate desirable membrane permeability for drug loading [41]. Electrical breakdown is achieved by membrane polarization for microseconds using varied voltage of 2kv/cm is applied for 20 µsec. The potential difference across the membrane is built up 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 A suitable procedure could be subsequently used to reseal these pores [42]. The various candidates entrapped by this method include primaquine and related 8–amino–quinolines, vinblastine, chlorpromazine and related phenothiazines, hydrocortisone, propranolol, tetracaine, and vitamin A [43, 44].

Entrapment by Endocytosis

This method was reported by Schrier et al. in 1975. Endocytosis involves the addition of one volume of washed packed erythrocytes to nine volumes of buffer containing2.5 mM ATP, 2.5 mM MgCl2, and1mM CaCl2, followed by incubation for2 min at room temperature. The pores created by this method are resealed by using 154 mM of NaCl and incubation at 37^0cfor 2 min. The entrapment of material occurs by endocytosis. The vesicle membrane separates endocytosed material from cytoplasm thus protecting it from the erythrocytes and vice-versa. The various candidates entrapped by this method include primaquine and related8–amino–quinolines, vinblastine, chlorpromazine and related phenothiazines, hydrocortisone, propranolol and tetracaine. [30-32]

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. 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. [33-34]

Loading by Lipid Fusion

Lipid vesicles containing a drug can be directly fused to human erythrocytes, which lead to an exchange with a lipid-entrapped drug. [35] This technique was used for entrapping inositol mono phosphate to improve the oxygen carrying capacity of cells. However, the entrapment efficiency of this method is very low (1%).

Use of Red Cell Loader

Magnani et al. developed a novel method for entrapment of non diffusible drugs into erythrocytes. They developed a piece of equipment called a "red cell loader". With as little as 50 mL of a blood sample, different biologically active compounds were entrapped into erythrocytes within a period of 2 h at room temperature under blood banking conditions. The process is based on two sequential hypotonic dilutions of washed erythrocytes followed by concentration with a hemofilter and an isotonic resealing of the cells. There was 30% drug loading with 35–50% cell recovery. The processed erythrocytes had normal survival in vivo. The same cells could be used for targeting by improving their recognition by tissue macrophages. [22]

IN-VITRO CHARACTERIZATION OF RESEALED ERYTHROCYTES

Drug content quantification

To determine the drug content, packed loaded cells are deproteinized with acetronitrile after centrifugation at 3000 rpm for a fixed time interval. The clear supernatant liquid is analysed spectrophotometrically [48].

In-vitro drug release and hemoglobin content study

In-vitro release of drug(s) and hemoglobin are monitored periodically form drug-loaded cells. The cells suspension (5% hematocrit in PBS) is stored at 4 ^{0}C in amber colored glass containers. Periodically the clear supernatant are withdrawn using a hypodermic syringes equipped with 0.45 µ filter, deproteinied using methanol and were estimated for drug content. The supernatant of each sample after centrifugation is collected and assayed, % hemoglobin release may be calculated using the formula.

% hemoglobin release = A540 of sample - A540 of background

A540 of 100% hemoglobin (Or)

Mean corpuscular hemoglobin {MCH (pg)} = Hemoglobin (g/100ml) x 10

Erythrocyte count (millions/cu mm)

Where a A540 refers to absorbance at 540nm [48].

Percent cell recovery and Morphological study

Percent cell recovery may be determined by counting the no. of intact cells per cubic mm of packed erythrocytes before and after loading the drug. Phase contrast or electron microscope may be used for normal and drug loaded erythrocytes

Osmotic shock

For 0.5 studies, erythrocyte suspension (1 ml, 10%) was diluted & centrifuge at 3000 rpm for 15 minute. The supernatant was estimated for % Hb release spectrophotometrically.

Turbulence shock

It is the measure of simulating distribution of loaded cells during injection. 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.

Entrapped magnetite study

The hydrochloric acid is added to a fixed amount of magnetite bearing erythrocytes and contents are heated at 600c for 2 hr. Then 20% w/v trichloro acetic acid is added and supernatant obtained after centrifugation is used to determine magnetite concentration using atomic absorption spectroscopy [49].

Self life and Stability and Cross linking of Released Erythrocytes

Glutaraldehyde (0.2%) treated erythrocytes in a sintered glass funnel (G-4) by filtration and dried in vacuum (200mm Hg) for 10 hr. Alternatively the erythrocyte suspension was filled into vials and lyophillized at- 40 0C to 0.01 torr using a laboratory lyophilizer. The dried powder was filled in amber color glass vials and stored at 4 0C for month. Improvement in shelf life of the carrier erythrocytes was achieved by storing the cells in powder from, ready for reconstitution at 4 0C.

Erythrocyte sedimentation rate (esr)

It is an estimate of the suspension stability of RBC in plasma and is related to the number and size of the red cells and to relative concentration of plasma protein, especially 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.

RELEASE CHARACTERISTICS OF LOADED DRUGS

There are mainly three ways for a drug to efflux out from the erythrocyte carriers: phagocytosis, diffusion through the membrane of the cells and using a specific transport system. RBCs are normally removed from circulation by the process of phagocytosis. The degree of cross linking determines whether liver or spleen will preferentially remove the cells. Carrier erythrocytes following heat treatment or antibody cross-linking are quickly removed from the circulation by phagocytic cells located mainly in liver and spleen. The rate of diffusion depends upon the rate at which a particular molecule penetrates through a lipid by layer. It is greatest for a molecule with high lipid solubility.

Delivery Strategies

As mentioned earlier, there are two major strategies in the delivery of drugs using erythrocytes as carriers which include intravenous slow drug release strategy and target gene delivery.

Intravenous slow drug release strategy

The normal life-span of an erythrocyte in systemic circulation is about 120 days. As mentioned as an advantage, in the optimum conditions of the loading procedure, the life-span of the resulting carrier cells may be comparable to that of the normal erythrocytes. [36] Erythrocytes have been used as circulating intravenous slow-release carriers for the delivery of antineoplasms, antiparasitics, antiretroviral agents, vitamins, steroids, antibiotics and cardiovascular drugs among others. [37-42]

A series of mechanisms have been proposed for drug release in circulation from carrier erythrocytes, including passive diffusion out of the loaded cells into circulation, specialized membrane-associated carriers, phagocytosis of the carrier cells by the macrophages of RES and, then, depletion of the drug into circulation, accumulation of the drug in RES upon lysis of the carrier and slow release from this system into circulation, accumulation of the carrier erythrocytes in lymphatic nodes following subcutaneous injection of the cells and drug release upon hemolysis in this sites, and, finally, haemolysis in the injection sites. [43]

Targeted drug delivery

RES or non-RES 'targeting' is another important strategy using erythrocytes as carriers.

RES targeting

It is a well-known fact that, in physiologic conditions, as a result of the gradual inactivation of the metabolic pathways of the erythrocyte by aging, the cell membrane loses its natural integrity, flexibility and chemical composition. These changes, in turn, finally result in the destruction of these cells upon passage through the spleen. The other effective site for the destruction of the aged or abnormal erythrocytes is the macrophages of the RES including peritoneal macrophages, hepatic Kupffer cells and alveolar macrophages of the lung, peripheral blood monocytes, and vascular endothelial cells. We know that aging and a series of other factors (e.g., stress during non-gentle loading methods) make the erythrocytes recognizable by the phagocyting macrophages via changing the chemical composition of the erythrocyte membrane, i.e., the phospholipids component. Therefore, a considerable fraction of carrier erythrocytes that have undergone some degrees of structural changes during the loading procedure will be trapped by the RES organs, mainly the liver and spleen, within a short time period after re-injection. [44-45]

A series of approaches have been evaluated to improve RES targeting using carrier erythrocytes. In one of these approaches, the drug-loaded erythrocytes have been exposed to membrane stabilizing agents. This may increase the targeting index of the erythrocytes to RES via decreasing the deformability of these cells. [46] [47]

Non-RES targeting

Recently, carrier erythrocytes have been used to target organs outside the RES. The various approaches include:

* Co-encapsulation of paramagnetic particles or photosensitive agents in erythrocytes along with the drug to be targeted;
* Application of ultrasound waves;
* Site-specific antibody attachment to erythrocyte membrane. Chiarantini et al. have reported in vitro targeting of erythrocytes to cytotoxic T-cells by coupling them to Thy-1.2 monoclonal antibody. [48] Price et al. reported the delivery of colloidal particles and erythrocytes to tissue through micro vessel ruptures created by targeted micro bubble destruction with ultrasound. In another study, the differential response of photosensitized young and old erythrocytes to photodynamic activation has been studied by Rollan. [49-50]

APPLICATIONS OF RESEALED ERYTHROCYTES

Resealed erythrocytes have several possible applications in various fields of human and veterinary medicine. Such cells could be used as circulating carriers to disseminate a drug within a prolonged period of time in circulation or in target-specific organs, including the liver, spleen, and lymph nodes. A majority of the drug delivery studies using drug-loaded erythrocytes are in the preclinical phase. In a few clinical studies, successful results were obtained. [51-61]

1. **It is used as Slow drug release**
   Erythrocytes have been used as circulating depots for the sustained delivery of antineoplastics, antiparasitics, veterinary antiamoebics, vitamins, steroids, antibiotics and cardiovascular drugs.
2. **It is used to Targeting the liver such as** Enzyme deficiency/replacement therapy, Treatment of hepatic tumors, Treatment of parasitic diseases.
3. **Removal of RES iron overload**
   Desferrioxamine-loaded erythrocytes have been used to treat excess iron accumulated because of multiple transfusions to thalassemic patients.
4. It is used to Removal of toxic agents
5. It is used as Enzyme Replacement Therapy in Gaucher's Disease
6. It is used to Delivery of antiviral agents such as azidothymidine derivatives, azathioprene, acyclovir, and fludarabine phosphate.
7. It is used to Improvement in oxygen delivery to tissues
8. It is used to Microinjection of macromolecules
9. Other applications of resealed erythrocytes include

* Surface modification with antibodies
* Surface modification with gluteraldehyde
* Surface modification with carbohydrates such as salicylic acid
* Entrapment of paramagnetic particles along with the drug
* Entrapment of photosensitive material
* Antibody attachment to erythrocyte membrane to get specificity of action

Table. No.1 -Various condition and centrifugal force used for isolation of erythrocytes

S.No	Species	Washing buffer	Centrifugal force(g)
1	Rabbit	10mmol KH2PO4/NaHPO4	50021000
2	Dog	15mmol KH2PO4/NaHPO4	50021000
3	Human	154mmol NaCl	<500
4	Mouse	10mmol KH2PO4/NaHPO4	1002500
5	Cow	10215mmol KH2PO4/NaHPO4	1000
6	Horse	2mmol MgCl2,10mmol glucose	1000
7	Sheep	10mmol KH2PO4/NaHPO4	50021000
8	Pig	10mmol KH2PO4/NaHPO4	50021000

Table.No.2 VARIOUS CHARACTERIZATION PARAMETERSAND THEIR DETERMINATION METHODS FOR RESEALED ERYTHROCYTES:

1. PHYSICAL PARAMETER

PARAMETER	METHOD /INSTRUMENT USED
Shape and surface morphology	Transmission electron microscopy, scanning electron microscopy, phase contrast microscopy, optical microscopy
Vesicle size and size distribution	Transmission electron microscopy, optical microscopy
Drug release	Diffusion cell, dialysis
Drug content	Deproteinization of cell membrane followed by assay of resealed drug, radiolabelling
Surface electrical potential	Zeta potential measurement
Surface Ph	pH-sensitive probes
Deformability	Capillary method

II. CELLULAR CHARACTERIZATION

% Hb content	Deproteinization of cell membrane followed by hemoglobin assay
Cell volume	Laser light scattering
% Cell recovery	Neubaur's chamber, hematological analyzer
Osmotic fragility	Stepwise incubation with isotonic to hypotonic saline solutions and determination of drug and hemoglobin assay
Osmotic shock	Dilution with distilled water and estimation of drug and hemoglobin
Turbulent shock	Passage of cell suspension through 30-gauge hypodermic needle at 10 ml/min flow rate and estimation of residual drug and hemoglobin, vigorous shaking followed by hemoglobin estimation
Erythrocyte sedimentation rate	ESR methods

III. BIOLOGICAL CHARACTERIZATION

Sterility	Sterility test
Pyrogenicity	Rabbit method, LAL test
Animal toxicity	Toxicity tests

SAFETY CONSIDERATIONS:

STEPS TO BE CONSIDERED	SAFETY ISSUE
Different blood types	Blood clotting
Possible risk of contamination	HIV,HBV etc.
Changing on physical and possible of clotting	Rigidity of membrane modification on erythrocytes membranes biochemical characteristics proteins lead to lysis. Extensive biotynilated leads to rapid elimination and kidney problems
Changing in pharmacokinetic and pharmacodynamic behaviors of loaded drug	Increase the production of unfavorable metabolites

Fig .No.1 Compositions of erythrocytes

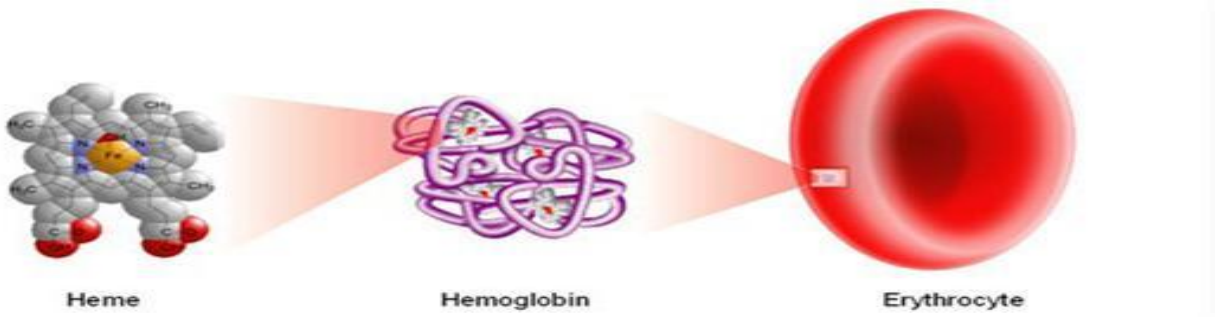
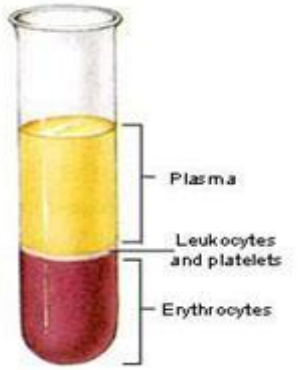


Fig .No.2 Isolation of Erythrocytes



Separation process	Isolated erythrocytes	Resealed erythrocytes

Fig .No.3 Methods of Drug Loading

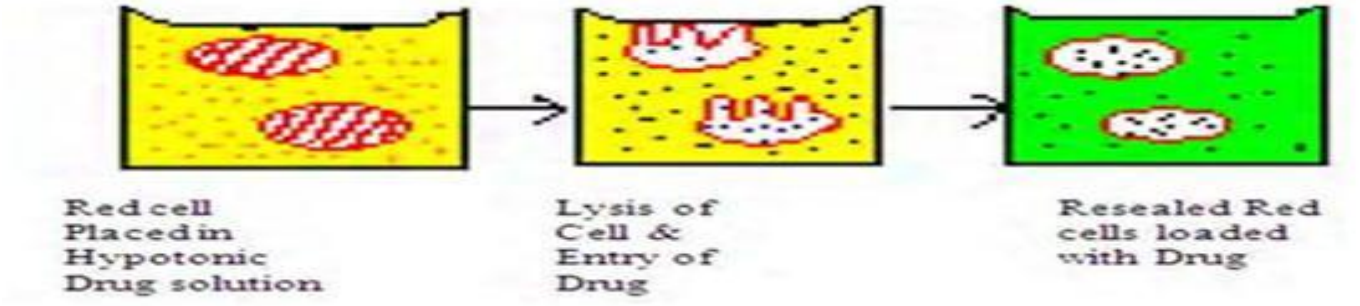


Fig .No.4 Schematic Illustration of Drug Loading in Erythrocytes

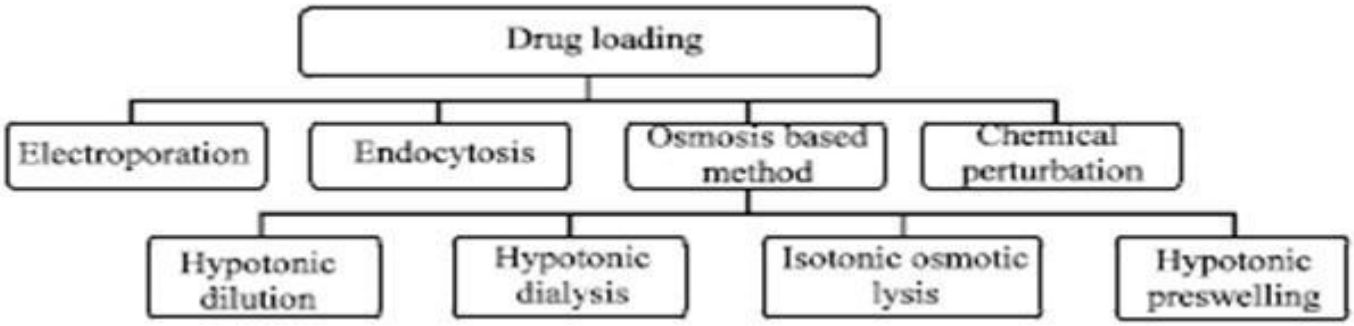


Fig. No.5 Hypotonic hemolysis

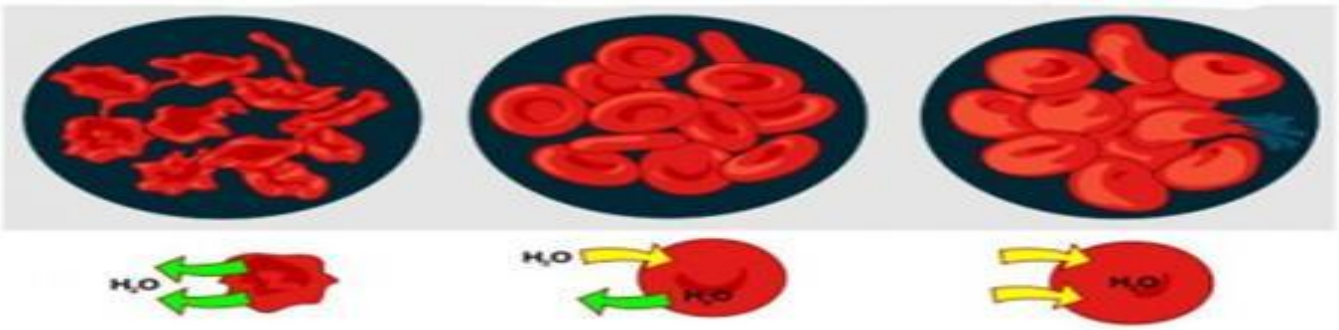


Fig.No.6 hypotonic press well method

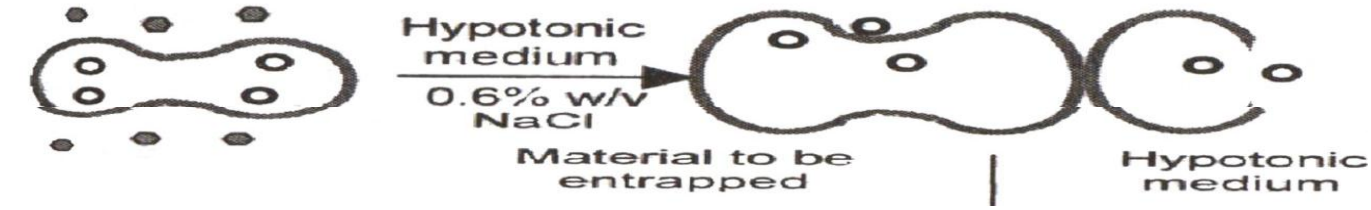


Fig.No.7 ISOTONIC OSMOTICLYSIS

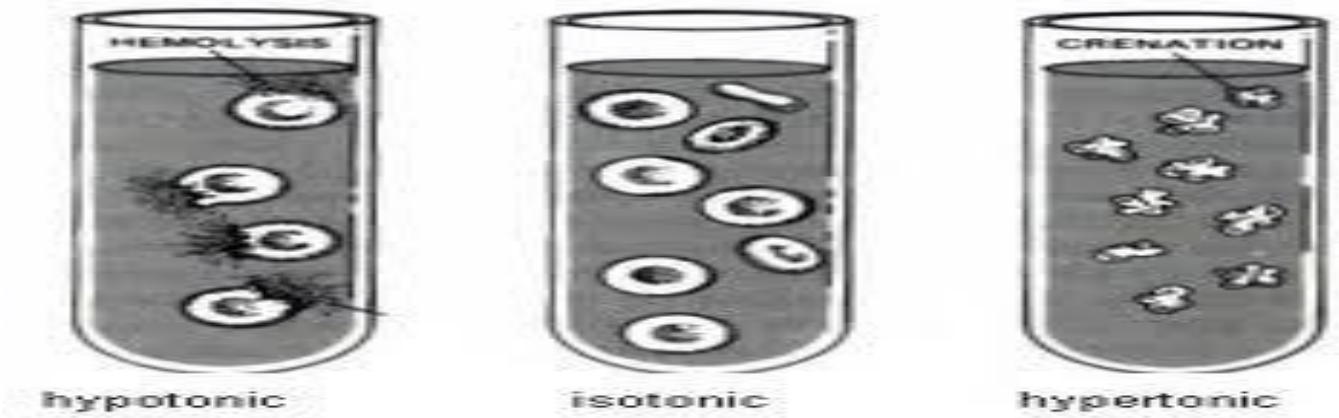


Fig.No.8 Dialysis method

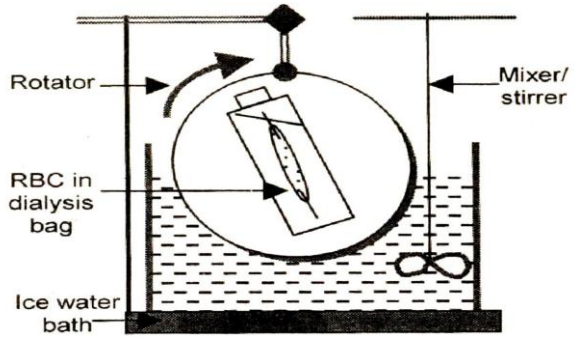


Fig.No.9 Electro-Insertion or Electro Encapsulation Method

Fig. A **Fig. A**

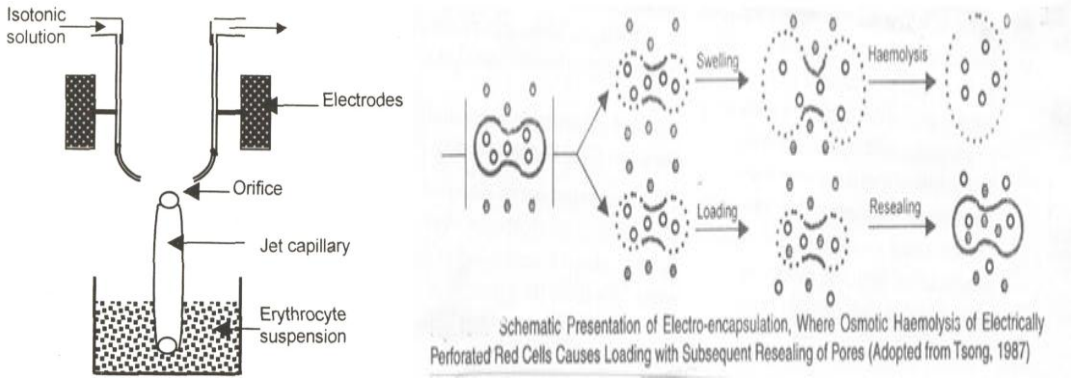


Fig.No.10 Entrapment by endocytosis method

Fig.No.11 Use of Red Cell Loader

CONCLUSION

During the past decade, numerous applications have been proposed for the use of resealed erythrocytes as carrier for drugs, enzyme replacement therapy etc. The use of resealed erythrocytes looks promising for a safe and sure delivery of various drugs for passive and active targeting. However, the concept needs further optimization to become a routine drug delivery system. The same concept also can be extended to the delivery of biopharmaceuticals and much remains to be explored regarding the potential of resealed erythrocytes. For the present, it is concluded that erythrocyte carriers are "golden eggs in novel drug delivery systems" considering their tremendous potential. Most of the studies in this area are in the in vitro phase and the ongoing projects worldwide remain to step into preclinical and, then, clinical studies to prove the capabilities of this promising delivery system.

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