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A REVIEW ARTICLE ON: RESEALED ERYTHROCYTES AS DRUG CARRIER

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ABSTRACT

Amongst the numerous carrier's rejected for aiming drugs to numerous body nerves, the cellular carriers encounter several standard's required in clinical applications, among the most important being biocompatibility of carrier as well its dilapidation products. Leucocytes, platelets, erythrocytes, Nano erythrocytes, hepatocytes, and fibroblasts etc. have been proposed as cellular carrier structures. Among these, the erythrocytes have been the most explored and have found to own better potential in drug delivery. Bio-pharmaceuticals, intensely significant peptides and proteins, antigens, anticancer drug as well as vaccines, are among the freshly attentive pharmaceuticals for being transported using carrier erythrocytes. Erythrocytes, better known as red blood cells, and have been broadly studied for their potential carrier capabilities for the delivery of drugs. The biocompatibility, non-pathogenicity, non-immunogenicity and biodegradability make them unique and useful carriers. Carrier erythrocytes are prepared by gathering blood sample from the organism of interest and separating erythrocytes from plasma. Via some methods, the cells are fragmented and the drug is hooked on the erythrocytes, finally they are resealed and the resulting carriers are then called as "resealed erythrocytes". So many drugs like aspirin, steroid, cancer drug which having many side effects are decrease by resealed erythrocyte. Current review highlights erythrocytes, drug loading methods, in vitro characterisation, application, advantages as well as disadvantages of resealed erythrocytes for drug delivery.

***INDEX TERMS*: Resealed Erythrocytes, carrier, drug loading, Applications.**

INTRODUCTION:

Current pharmaceutical scenario is aimed at development of drug delivery systems which make the most of the drug targeting along with high therapeutic benefits for safe and effective management of diseases [1]. Targeting of an active biomolecule from active drug delivery where pharmacological moderator directed definitely to its goal site. Drug targeting can be methods by either chemical modification otherwise by suitable carrier [2]. Numerous carriers has been used for the drug targeting amongst which cellular carrier offer a better potential benefits associated to its biodegradability, non-pathogenicity, non-immunogenicity, biocompatibility as well as self-degradability along with high drug loading efficiency [3]. Leukocytes, platelets and erythrocytes have been proposed as cellular carrier organizations [4]. Blood contains different type of cells like erythrocytes (RBC), leucocytes (WBC) and platelets, among them erythrocytes are the most interesting carrier and possess excessive potential in drug delivery due to their capability to circulate all over in the body[5]zero order kinetics, reproducibility and ease of preparation primary aim for the development of this drug delivery system is to maximize therapeutic performance, reducing undesirable side effects of drug and increase patient obedience. [6]

ERYTHROCYTES

Red blood cells are the most communal kind of blood cells and the vertebrate organism's main resources of delivering oxygen (O2) to the body tissues through the blood flow through the circulatory system. They receipt up oxygen in the lungs or gills and release it while squeezing via the body's capillaries. These cells' cytoplasm is rich in haemoglobin, an iron-containing biomolecule that can bind oxygen and is responsible for the blood's red colour. In humans, developed red blood cells are flexible biconcave disks that absence a cell nucleus and most organelles. 2.4 million new erythrocytes are produced per second [7].

ANATOMY AND PHYSIOLOGY OF RBCS

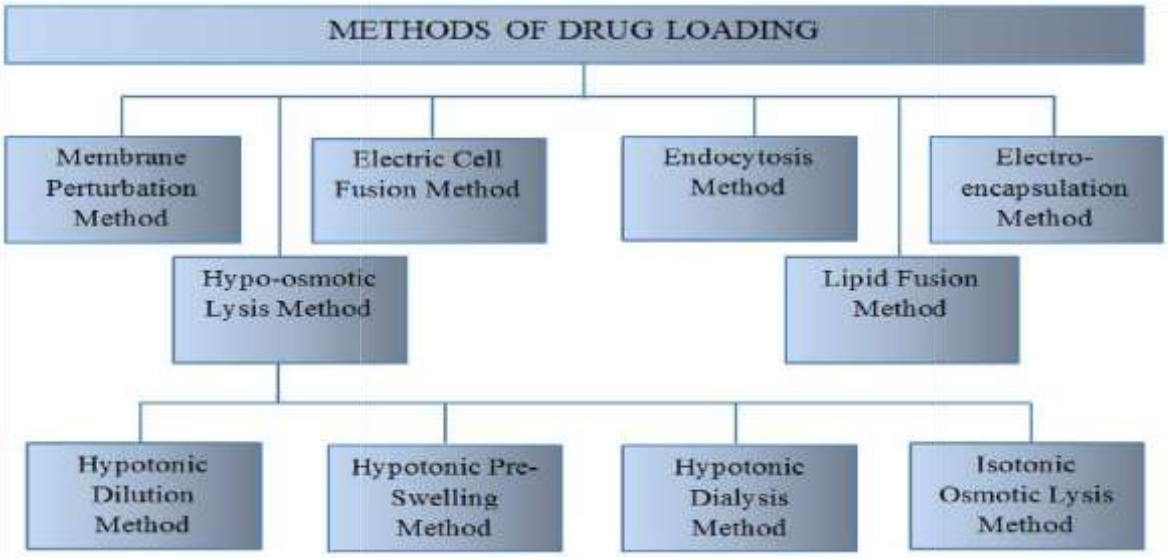
RBCs have shapes similar to biconcave discs with a diameter of 7.8 μm and width near 2.2 μm. Mature erythrocytes have a modest structure. It is as well elastic in nature. Their plasma membrane is both strong as well as flexible, which permits them to deform lacking of rupturing as they squash through narrow capillaries. RBCs lack a nucleus and other organelles and can neither replicate nor carry on wide metabolic activities. RBCs are highly dedicated for their oxygen transport function, as their mature RBCs have no nucleus, all their internal space is offered for oxygen transport. Even the shape of RBC services it's function. A biconcave disc has a much better surface area for the diffusion of gas molecules into and out of the RBC than would; say a sphere or a cube. The red blood cell membrane, a dynamic, semi permeable components of the cell, associated with energy metabolism in the maintenance of the permeability characteristic of the cell of various cations (Na^+, K^{++}) and anions (Cl^- HCO_3^-). Each RBC contains about 280 million haemoglobin molecules. A haemoglobin molecule consists of a protein called globin, composed of four polypeptide chains; a ring like non-protein pigment called a heme, is bound to each of the four chains. At the centre of the heme ring combine reversibly with one oxygen molecule, allowing each haemoglobin molecule to bind four oxygen molecules. RBCs include water (63%), lipids (0.5), glucose (0.8%), mineral (0.7%), non-haemoglobin protein (0.9%), meth haemoglobin (0.5%), and haemoglobin (33.67%). [6] [9] [10]

RESEALED ERYTHROCYTES

This drug-loaded transporter RBC`s are prepared simply by gathering blood models from the organism of choice, separating erythrocytes from plasma, entrapping drug within the erythrocytes, as well as resealing the resulting cellular carriers [11]. Hence, these carriers are called resealed erythrocytes. The complete process is based on the response of these cells under osmotic conditions. Upon re-injection, the drug-loaded erythrocytes assist as slow circulating depots and target the drugs to a reticuloendothelial system. [12]

METHODS OF DRUG LOADING

several techniques can be used to load drugs or other bio-active complexes in erythrocytes, like physical method (e.g., electrical pulse method) also chemical methods like (e.g., chemical perturbation of the erythrocytes membrane). Irrespective of the method used, the optimal characteristics for the successful entrapment of the compound requires the drug to have a considerable degree of water solubility, resistance in contrast to degradation in erythrocytes, lack of physical or chemical interaction with erythrocyte membrane, and precise pharmacokinetic and pharmacodynamic properties [13].



1.HYPOTONIC DILUTION METHOD: Hypotonic dilution was the first method explored for the encapsulation of chemicals into erythrocytes and is the simplest as well as fastest. In this technique, a volume of packed erythrocytes is diluted with 2–20 volumes of aqueous solution of a drug. The solution tonicity is then restored by addition a hypertonic buffer. The resultant mixture is then centrifuged, the supernatant is cast-off, and the pellet is washed by means of isotonic buffer solution. The major drawbacks of this technique include low entrapment efficacy and a considerable loss of haemoglobin and other cell components. This reduces the circulation half-life of the loaded cells. These cells are willingly 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, asparaginase, and arginase, as well as bronchodilators for example salbutamol.

2.HYPOTONIC PRE-SWELLING METHOD: It is based upon initial controlled swelling in a hypotonic buffered solution. This mixture is centrifuged at small *g* values. The supernatant is castoff and the cell fraction is carried to the lysis point by adding 100–120 µL portions of an aqueous solution of the drug to be encapsulated. After that the mixture is centrifuged between the drug-addition steps. The tonicity of a cell mixture is reinstated at the lysis point by adding a planned amount of hypertonic buffer. The lysis point is noticed by the disappearance of a distinct boundary between the cell fraction and the supernatant upon centrifugation. Finally the cell suspension is incubated at 37 ^{0}C to reanneal the resealed erythrocytes [15,16,17].

3.HYPOTONIC DIALYSIS METHOD: This method was firstly reported by Klibansky in 1959 and was used in 1977 by Deloach and Ihlerand Dale for loading of enzymes as well as lipids. There are many procedures were based on the principle of semi permeable dialysis membrane which exploits the intracellular, extracellular volume ratio for macromolecules throughout lysis and resealing. A desired hemocrit is attains in this course by mixing of erythrocyte suspension and drug solution. The mixture is hired into dialysis tubing and then both ends of tube are tied by thread. An air bubble of almost 25% of the internal volume is left in the tube. The tube were placed in the bottle containing 100ml of swelling solution. The bottle is placed at 4oC for the demand lysis time. The contents of the dialysis tubing are mixed by means of shaking the tube using the strings. Then dialysis tube is placed in 100 ml of resealing solution. After that the loaded erythrocytes thus obtained, then washed with cold phosphate buffer at 4°C. A good entrapped efficacy is found in this [18,19]. It has been used for loading enzymes like β-galactosidase, glucocerebrosidase, asparaginase, inositol hex phosphatase as well as drugs such as gentamicin, Adriamycin, pentamidine and duramycin, interlukin-2, deferoxamine and human recombinant erythropoietin [20].

4.ISOTONIC OSMOTIC LYSIS METHOD: It is also called as the osmotic pulse method. If erythrocytes are incubated in solutions of a substance with high membrane permeability, the solute will diffuse into the cells because of the concentration gradient and it is followed by an influx of water to sustain osmotic equilibrium. Chemicals together with urea solution, polyethylene glycol also ammonium chloride have been used for isotonic haemolysis. Lastly, suspension was diluted with isotonic-buffered drug solution and cells were separated & resealed at 37^{0}C [21].

5.MEMBRANE PERTUBRATION METHOD: This technique is based on the rise in membrane permeability of erythrocytes when the cells are exposed to typical chemicals. In 1973, Deuticke et al. exposed that the permeability of erythrocytic membrane rises upon exposure to polyene antibiotic such as amphotericin B [22]. In 1980, this technique was used successfully by Kitao and Hattori to entrap the antineoplastic drug daunomycin in human and mouse erythrocytes [23]. Lin et al. used halothane for the similar purpose. However, these procedures induce irreversible destructive changes in the cell membrane and hence are not very popular.[24]

6.ELECTRO-ENCAPSULATION METHOD: It is also recognized as electroporation method, which is based on using transient electrolysis leading to generate pores that produce desirable membrane permeability for drug loading into erythrocytes. It includes suspending of erythrocytes in an isotonic buffer in an electrical discharge chamber. It has a capacitor in an exterior circuit which is charged to a certain voltage and then discharged within a definite time interval through cell suspension to produce a square-wave potential. In 1980, it was successfully used to entrap the antineoplastic drug daunomycin in human and mouse erythrocytes. This technique also induces irreversible destructive changes in the cell membrane and henceforward is not very popular [25,26,27].

7.ENDOCYTOSIS METHOD: It was reported by Schrier in 1975. Endocytosis includes the addition of one volume of washed packed erythrocytes to nine volumes of buffer holding 2.5 mM ATP, 2.5mM $MgCl_2$ and 1mM $CaCl_2$ tracked by incubation for 2 min. at room temperature. The pores formed by this method are resealed by using 154 mM of NaCl and incubation at 37°C for 2 min. The vesicle membrane splits endocytosed material from cytoplasm & protecting it from the erythrocytes. Various drugs are used in this method primaquine, 8aminoquinolones, vinblastine, chlorpromazine, phenothiazine, hydrocortisone, tetracaine, & vitamin A [28,29].

8.LIPID FUSION METHOD: Lipid fusion method involves lipid vesicle containing a drug can be directly fused to human erythrocytes; it leads to an exchange with a lipid-entrapped drug. Hence this method is used for entrapping inositol monophosphate which helps to improve the O_2 carrying capacity of RBCs [29].

9.ELECTRIC CELL FUSION METHOD: This method includes the early loading of suspended from the commencement of the experimentation. The typical pore diameter created in the membrane depends upon the intensity of electric field, the discharge time, and the ionic strength of suspending medium [30,31,32]. The colloidal macromolecules contents of the cell may lead to cell lysis because of the increase in osmotic pressure. This process can be prevented by adding large molecules (e.g. tetra saccharide stachyose and bovine serum albumin) and ribonuclease. One advantage of this method is a more uniform distribution of loaded cells in comparison with osmotic methods [33]

ROUTES OF ADMINISTRATION: It was reported that survival of cells in circulation to the cells administered by intra peritoneal injection is equivalent to iv, injection. The subcutaneous route for measured release of entrapped agent, reported that the loaded cell released encapsulated molecules at the injection site. Generally resealed erythrocyte throughout experimentation has been administered to the laboratory animals intravenously through cardinal vein. The scientist De Loach used subcutaneous route for slow release of entrapped mediators and assessed the disposition of the interleukin-2 in mice receiving a subcutaneous injection. Talwar (1993) have been planned erythrocyte based nasal delivery of propranolol [34,35].

In vitro characterization: The in-vitro performance of resealed erythrocytes is pretentious to a great extent by their biological goods. Hence, in vitro characterization forms an significant part of studies involving such cellular carriers. Table I reviews the various evaluation parameters and the techniques applied for their determination.

1.The morphology of erythrocytes decides their life span after administration.

Light microscopy reveals no noticeable change in resealed cells [36,37] but in few cases spherical erythrocytes (spherocytes) are spotted [38,39]. Scanning electron microscopic studies have shown that a majority of the cells uphold their biconcave discoid shapes after the loading process [40], and few stomatocytes—a form of spherocytes with an invagination in one point are formed [41]. In some cases, cells of smaller size (microcyte) are also observed [42].

2.Shape change (deformability) is another factor that affects the life span of the cells.

This parameter evaluates the effortlessness of passage of erythrocytes through slim capillaries and the RES. It determines the rheological behaviour of the cells and depends on the viscoelasticity of the cell membrane, viscosity of the cell contents, and the cellular surface-to-volume ratio [43]. The deformability is restrained by passage time of certain volume of cells through capillary of 4 m diameter or polycarbonate filter with typical pore size of 45 m [43,44]. Another indirect approach is to evaluate chlorpromazine induced shape changes turbidimetrically [45].

3.Drug content of the cells determines the entrapment efficiency of the method used.

The procedure involves deproteinization of packed, loaded cells (0.5 mL) with 2.0 mL acetonitrile and centrifugation at 2500 rpm for 10 min. The clear supernatant is analysed for the drug content [46].

The most significant parameters for evaluation of resealed erythrocytes is the drug release pattern. Haemoglobin is too invariably released because drug release includes the loss of cell membrane integrity indicating haemolysis. On the basis of the several in-vitro release experiments carried out on these cells, three general drug release patterns are detected: The rate of drug release is considerably higher than that of 148

4. Haemoglobin In other words, drug diffuses readily.

Such a pattern is shown by lipophilic drugs, including methotrexate [47], phenytoin dexamethasone [48], primaquine [49], and vitamin B_{12} [50]. Cell lysis is not important for the release of such drugs.

5. The rate of drug release is analogous to that of haemoglobin.

This shows that cell lysis is essential for drug release and drug can-not be released by mere diffusion. Polar drugs for example gentamicin [51,52], heparin [51], and enalaprilat [53], and enzymes such as asparaginase [54,55,56,57], peptides, including progesterone and l-lysine-l-phenylalanine [58] follow such pattern.

APPLICATIONS OF RESEALED ERYHROCYTES AS DRUG CARRIER

IN-VIVO APPLICATIONS

1.Slow drug release: Erythrocytes have been used as circulating depots for the sustained delivery of antineoplastic, antiparasitic, veterinary anti-amoebic, vitamins, steroids, antibiotics and cardiovascular drugs device contain for drug release is gathering of erythrocytes in lymph nodes upon subcutaneous administration trailed by haemolysis to release the drugs [59,60].

2)Drug targeting: Drug delivery should be site definite and target oriented to exhibit best therapeutic index with least adverse effects. It acts as drug carriers also targeting tools as well. It can be used to target RES organs as well as non-RES organs. To target organs of mononuclear phagocytic organization/ RES Surface modified erythrocytes are used because the change in the membrane is known by macrophages [61,62].

3)Treatment of hepatic tumours: Antineoplastic drugs like methotrexate, bleomycin, asparaginase also Adriamycin have been effectively delivered by erythrocytes to cure hepatic tumours. Daunorubicin diffuse speedily from the cells upon loading and hence shows a problem which can be overwhelmed by covalently linking daunorubicin to the erythrocytic membrane using glutaraldehyde orcisaconitic acid as a spacer [63].

4)Removal of RES iron overloads: To treat excess iron gathered deferoxamine loaded erythrocytes have been used since, of several transfusions to thalassaemic patients. This drug targeting to the RES is very helpful because the aged erythrocytes are destroyed in RES organs, which results in an accretion of iron in these organs [64].

5)Carriers for enzymes: For this purpose enzymes can be vaccinated into the blood stream to substitute a missing or absent enzyme in metabolic disorders or to reduce toxic compounds accumulated in the blood due to a disease such as, environmental, lysosomal storage disorders such as Gaucher's disease, hyperargininaemia, hyperuricaemia, hyperphenylalaninemia and kidney failure are only few examples of metabolic complaints that can be treated by supervision of enzymes [65].
Phagocytosis cells have been used for *in vitro* to simplify the uptake of enzymes by phagolysosomes. The enzyme content inside carrier RBC could be visualized with the help of cytochemical method. The most frequent in vitro application of RBC is that of micro-injection in which protein or nucleic acid was injected into eukaryotic cells by fusion process. Similar in case of antibody, where antibody molecules are presented using erythrocytic carrier system & it instantly diffuse throughout the cytoplasm [66]

NOVEL APPROACHES

a)Nanoerythrosomes

An erythrocyte based new drug carrier, named nano-erythrosome has been established which is prepared by extrusion of erythrocyte flickers to produce small vesicles having an average diameter of 100 nm. Daunorubicin (DNR) was co-valently conjugated to the n-Eryt (nErytDNR) by means of glutaraldehyde as homodifunctionalized linking support. This led to a complex that is extra active than free DNR both in vitro and in vivo. Daunorubicin (DNR) conjugated to these nano-erythrosomes has a advanced antineoplastic index than the allowed drug. Moreover, since nanoerythrosomes are particles, phagocytosis may be elaborate in their machinery of potentiation.[68]

b) Erythrosomes

These are specially engineered vesicular systems that are chemically cross-linked to human erythrocytes' provision upon which a lipid bilayer is coated. This process is achieved by modifying a reverse-phase evaporation technique. These vesicles have been proposed as useful encapsulation schemes for macromolecular drugs.[69]

ADVANTAGES

1.Biocompatible, particularly when autologous cells are used hence no possibility of triggered immune response.
2.Biodegradability with no generation of toxic products.
3.Considerable uniform size and shape of carrier.
4.Relatively inert intracellular environment can be encapsulated in a small volume of cells.
5.Isolation is easy and large amount of drug can be loaded.
6.Prevention of degradation of the loaded drug from deactivation by endogenous chemical.
7.Entrapment of wide variety of chemicals can be possible.
8.Entrapment of drug can be possible without chemical modification of the substance to be entrapped.
9.Possible to maintain steady-state plasma concentration, reduce fluctuation in concentration.
10.Protection of the organism against toxic result of drug.
11.Targeting to the organ of the RES.
12.Ideal zero-order drug release kinetic.
13.Prolong the systemic activity of drug by residing for a longer time in the body.[70-79]

DISADVANTAGES

1.They have a limited potential as carrier to non-phagocyte target tissue. [80]
2.Possibility of clumping of cells and dose dumping may be there [81]

CONCLUSION

The use of resealed erythrocytes looks hopeful for a safe and sure delivery of several drugs for passive and active targeting. However, the idea needs further optimization to become a routine drug delivery system. The Resealed erythrocytes had also been employed for effective delivery of numerous drugs as showed by many researchers for the treatment of cancer, tumour, and arthritis and also for operative treatment of the poisoning. However, in near future, erythrocytes-based delivery system with their ability to provide controlled and site-specific drug delivery will revolutionize in effective treatment of various disease. For the present, it is concluded that erythrocyte carriers are "Nano Device in field of Nanotechnology" considering their tremendous potential and prospective.

FiguresandTables:

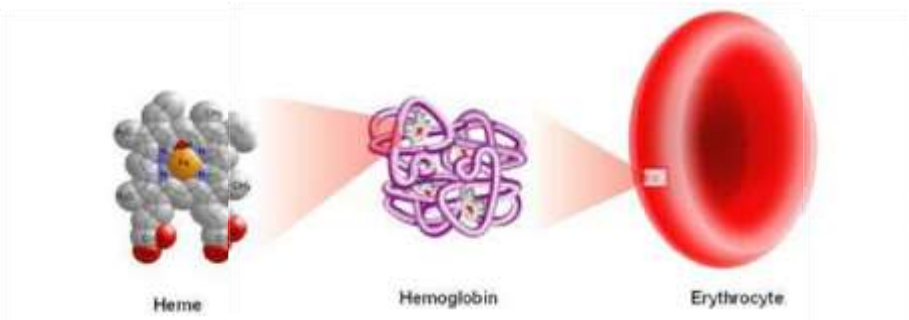


Figure No 1: Introduction of Erythrocytes [8]

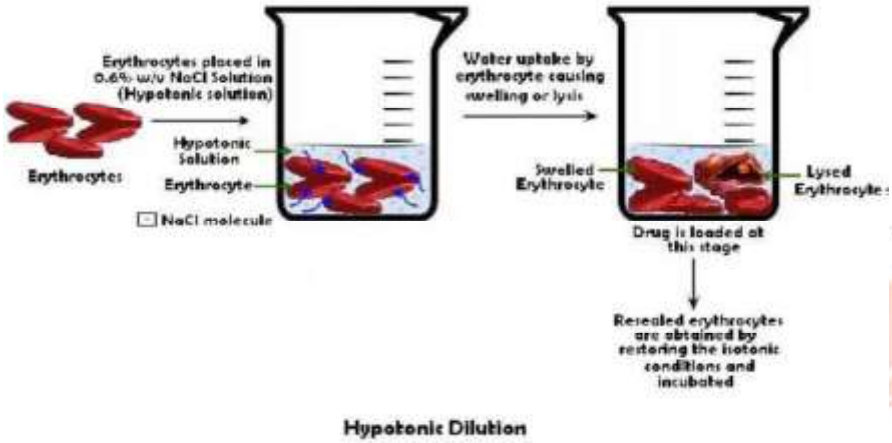


Figure no. 2: Hypotonic Dilution method

Figure.no.3: Hypotonic pre-swelling

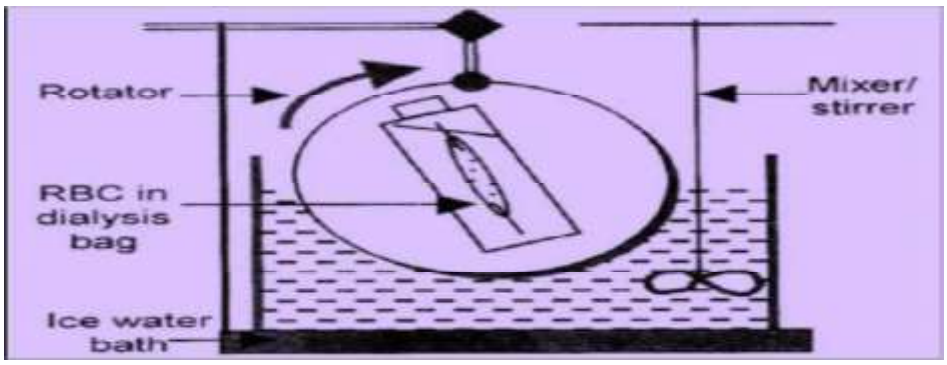


Figure.4: Hypotonic Dialysis technique

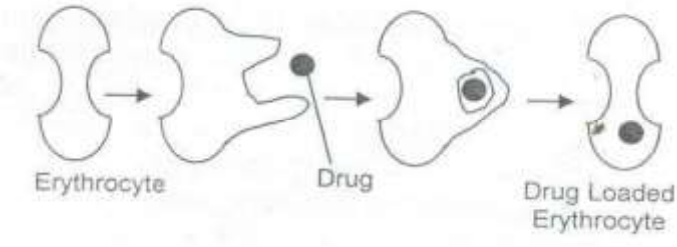


Fig. no. 5: endocytosis method

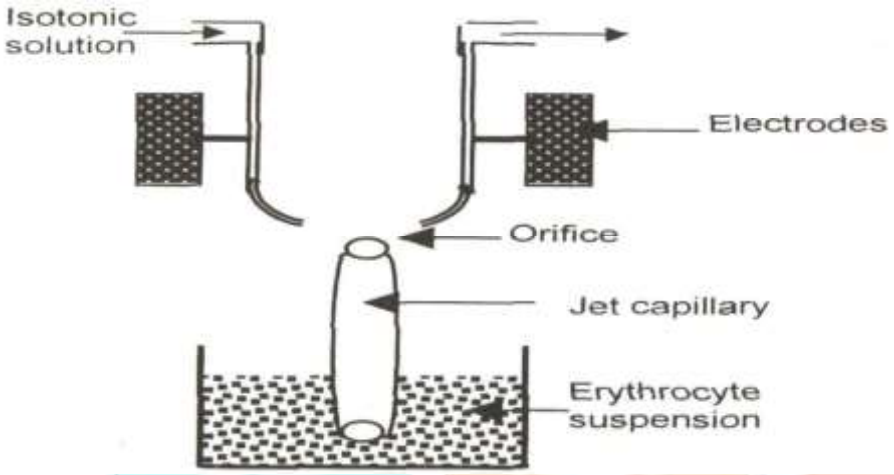


Figure.6: Lipid diffusion method

Table no. 1 summary of characterization parameters and their determination for resealed erythrocytes.

Table I: Summary of characterization parameters and their determination for resealed erythrocytes

Parameter	Method/instrument used
I. Physical characterization	
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 haemoglobin assay
Cell volume	Laser light scattering
% Cell recovery	Neubauer's chamber, haematological analyser
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 haemoglobin, vigorous shaking followed by haemoglobin estimation
Erythrocyte sedimentation rate	ESR methods
III. Biological characterization	
Sterility	Sterility test
Pyrogenicity	Rabbit method, LAL test

Table no. 2: Resealed Erythrocytes in RES Targeting [67]

Treatment/Diseases	Name of Drug(s)	Purpose
Lysosomal storage diseases	C-glucuronidase, Lysosomal enzymes, 13-galactosidase and 6-giucosidase	It is used to deliver lysosomal enzymes and drugs to lysosomes of the erythrophagocytosis cells.
Gaucher's Disease	Glucocerebrosidase	By using this loaded cells survived for 10 days in treated patient and no adverse reactions were found with respect to blood counts, blood pressure and renal functions.
Liver tumours	Anticancer drugs like Bleomycin, Adriamycin, Carboplatin, Gentamycin, etc.	Used to target hepatic carcinomas
Parasitic Diseases	immunoglobulin-G coated erythrocytes, Pentamidine loaded	treatment of parasitic diseases by targeting drug in which the parasite resides in the organs of RES.
Removal of toxic substances	Murine carrier erythrocytes containing bovine rhodanese thiosulphate	Used to antagonize the lethal effects of potassium cyanide in mice or antagonism of cyanide intoxication.

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REFERANCES:

[1]. Gopal V. S., Doijad R.C., and Deshpande P. B. Erythrocytes as a carrier for prednisolone- in vitro and in vivo evaluation., Pak J. Pharm. Sci, 2010 (2) ; 23: 194-200.

[2]. Sawant K.K., Soni H.N. and Murthy R. S. R.,Investigation on resealed erythrocytes as carrierfor 5flurouracil, Indian J. Pharm. Sci. 2001(2); 63: 105-109.

[3] Nicholas B., Retrometabolic approaches to drug targeting membrane and barrier In : Rapaka RS (editor), NIH Publication, 1995: 1-6

[4] Eicher H.G. and Ramies H., Survial of Gentamicine loaded carrier erythrocytes in healthy human volunteers. Eur. J. Clin. Invest. 1986 (1); 16: 39-42.

[5].Singh Devendra, Kumar Manish, Singh Talever, Singh L.R., Singh Dashrath. A Review on Resealed Erythrocytes as a Carrier for Drug Targeting, International Journal of Pharmaceutical And Biological Archives 2011; 2 (5):1357-1373.

[6]. Patel R P, Patel MJ and Patel A. An overview of resealed erythrocytes drug deliver, R.P. Patel, journal of pharmacy research 2009; 2(6):1008-1012.

[7].Sackmann Erich, Biological Membranes Architecture and Function., Handbook of Biological Physics, (ed. R.Lipowsky and E.Sackmann, vol.1, Elsevier, 1995.

[8]. Shyama SK., Rathore KS., Keshri R., Resealed Erythrocytes: Potential Carrier for delivery of Drugs, International Journal of Pharmaceutical Eruditon 2015;4:4:34-47.

[9]. G. J. Tortara B. Derrickson. The Cardiovascular System the Blood in Principles of Anatomy and Physiology, New York, NY, 7th ed., 1993:669-672.

[10]. Guyton A C and Hall JE. Red Blood Cells, Anemia and Polycytemia, in test book of medical physiology, Saunders WB, Philadelphia, PA, 1996: 425-433.
[11]. Ropars C., Chassaigne M., and Nicoulau C. Advances in the Biosciences, Pergamon Press, Oxford, 1987: 67.
[12]. Sackmann Erich, Biological Membranes Architecture and Function Handbook of Biological Physics, ed. R.Lipowsky and E.Sackmann Elsevier 1995: 1.
[13]. M. Hamidi and H. Tajerzadeh,"Carrier Erythrocytes: An Overview,"*Drug Delivery* **10,** 9–20 (2003).
[14] Suresh Rewar, BK Bansal, CJ Singh, *International Journal of Urgent Research in Chemistry Science*, **2014**, 101-114
[15]. TV Thulasiramaraju, A Arunachalam, GV Surendra babu, N Syamkumar, VV Nagendra babu, M Nikilesh babu, *International Journal of Preclinical and Pharmaceutical Research* , **2011**, 3-13.
[16]. A V Gothoskar, *Pharma. Tech. com*, **2004**, 140-158.
[17]. G M Ihler, HCW Tsang, *Methods Enzymol.*, **1987**, 149, 221– 229.
[18]. E Venkatesh, C Aparna, K Umasankar, P Jayachandra Reddy, V Prabhakaran, *Int. J. Pharm. Sci. Rev. Res.*, **2013**, 23(2), 298-306.
[19]. R D Amrutkar, TG Vyawahare, RS Bhambar, *International Journal of Pharmaceutical Research*, **2011**, 3(3).
[20]. Suresh Rewar, BK Bansal, CJ Singh, *International Journal of Urgent Research in Chemistry Science*, **2014**, 101-114.
[21]. Shashank shah, *International journal of pharma & bioscience*, **2011**, 2 (1), 394-406.
[22]. B. Deuticke, M. Kim, and C. Zolinev,"The Influence of AmphotericinB on the Permeability of Mammalian Erythrocytes to Nonelectrolytes, anions and Cations," *Biochim. Biophys. Acta.* **318,** 345–359 (1973).
[23]. T. Kitao, K. Hattori, and M. Takeshita, "Agglutination of Leukemic Cells and Daunomycin Entrapped Erythrocytes with Lectin In Vitro and In Vivo," *Experimentia* **341,** 94–95 (1978).
[24]. W. Lin et al.,"Nuclear Magnetic Resonance and Oxygen Affinity Study of Cesium Binding in Human Erythrocytes," *Arch Biochem Biophys.* **369,** (1) 78–88 (1999)
[25]. Pragya, V Rastogi, *International Journal of Pharmacy and Pharmaceutical Sciences*, **2012**, 4(3), 75-82.
[26]. M Hamidi, N Zarei, M Foroozesh, Mohammadi Samani S. *J. Control Release*, **2007**, 118: 145-160.
[27]. A Kumar, M verma, KK Jha, *The Pharma Innovation*, **2012**, 1(2), 7-15. 4.'
[28]. AK Shah, A Rambhade, A Ram, SKJ ain, *Journal of chemical & pharmaceutical research*, **2011**, 3(2). [29]. Rajendra Jangde, *Asian J. Res. Pharm. Sci.*, **2011**, 1(4), 83-92.
[30]. C. Ropars M. Chassaigne, and C. Nicoulau, *Advances in the BioSciences*, (Pergamon Press, Oxford, 1987), p. 67.
[31]. K. Kinosita and T.Y. Tsong,"Survival of Sucrose-Loaded Erythrocytesin the Circulation," *Nature* **272,** 258–260 (1978).
[32]. K. Kinosita and T.Y. Tsong,"Formation and Resealing of Pores of Controlled Sizes in Human Erythrocyte Membrane,"*Nature* **268,** 438–441 (1977).
[33]. U. Zimmermann, *Cellular Drug-Carrier Systems and Their Possible Targeting In Targeted Drugs*, EP Goldberg, Ed. (John Wiley & Sons, New York, 1983), pp. 153–200.
[34]. D Raut, RS sakhare, KD Ketan, PD Halle. *IJRPC*, **2013**, 3(2), 198-207.
[35]. E Venkatesh, C Aparna, K Umasankar, P Jayachandra Reddy, V Prabhakaran, *Int. J. Pharm. Sci. Rev. Res.*, **2013**, 23(2), 298-306.
[36]. N. Talwar and N.K. Jain,"Erythrocytes as Carriers of Primaquin Preparation: Characterization and Evaluation," *J. Controlled Release* **20,** 133–142 (1992
[37]. J.R. Deloach and G.M. Ihler,"A Dialysis Procedure for Loading of Erythrocytes with Enzymes and Lipids," *Biochim. Biophys. Acta.* **496,** 136–145 (1977).
[38]. S. Jain, S.K. Jain, and V.K. Dixit,"Erythrocytes Based Delivery of Isoniazid: Preparation and In Vitro Characterization," *Indian Drugs* **32,** 471–476 (1995).
[39]. S. Jain, S.K. Jain, and V.K. Dixit, "Magnetically Guided Rat Erythrocytes Bearing Isoniazid: Preparation, Characterization, and Evaluation," *Drug Dev. Ind. Pharm.* **23,** 999–1006 (1997.
[40]. J.R. Deloach and R. Doleskey,"Preparation and Properties of Microcytic Carrier Erythrocytes from Sheep and Goats,"*Adv. Biosci.* (series) **67,** 199–212 (1987).
[41]. M. I. Garin et al.,"Erythrocytes as Carriers for Recombinant HumanErythropoietin," *Pharm. Res.* **13,** 869–874 (1996).
[42]. B. Teisseire et al.,"In Vivo Consequences of Rightward Shift of the Hemoglobin Dissociation Curve,"*Adv. Biosci.* (series) **67,** 89–94 (1987).
[43]. M. Jrade et al.,"Rheological Approach to Human Red Blood Cell Carriers Desferrioxamine Encapsulation," *Adv. Biosci.* (series) **67,** 29–36 (1987).

[44]. M. Jrade et al.,"Rheological Approach to Human Red Blood Cell Carriers Desferrioxamine Encapsulation," *Adv. Biosci.* (series) **67,** 29–36 (1987).
[45]. M. Hamidi et al., "In Vitro Characterization of Human Intact Erythrocytes Loaded by Enalaprilat," *Drug Delivery* **8,** 231–237 (2001).
[46]. V. Jaitely et al., "Resealed Erythrocytes: Drug Carrier Potentials andBiomedical Applications," *Ind. Drugs* **33,** 589–594 (1996).
[47] D.A. Lewis and H.O. Alpar, "Therapeutic Possibilities of Drugs Encapsulated in Erythrocytes," *Int. J. Pharm.* **22,** 137–146 (1984).
[48]. H.C. Eichler et al.,"In Vitro Drug Release From Human Carrier Erythrocytes," *Adv. Biosci.* (series) **67,** 11–15 (1987).
[49].N. Talwar and N.K. Jain,"Erythrocytes as Carriers of Primaquin Preparation: Characterization and Evaluation," *J. Controlled Release* **20,** 133–142 (1992).
[50]. H.G. Eichler et al.,"Release of Vitamin B_{12} from Carrier Erythrocytes In Vitro," *Res. Exp. Med.* **185,** 341–344 (1985).
[51]. H.C. Eichler et al.,"In Vitro Drug Release From Human Carrier Erythrocytes," *Adv. Biosci.* (series) **67,** 11–15 (1987).
[52]. H.G. Eichler et al.,"In Vivo Clearance of Antibody-Sensitized Human Drug Carrier Erythrocytes,"*Clin. Pharmacol. Ther.* **40,** 300–303 (1986).
[53]. G.M. Ihler,"Erythrocyte Carriers,"*Pharmacol.Ther.* **20,** 151–169 (1983).
[54]. V. Jaitely et al., "Resealed Erythrocytes: Drug Carrier Potentials andBiomedical Applications," *Indian Drugs* **33,** 589–594 (1996).
[55]. H.O.Alpar and D.A. Lewis,"Therapeutic Efficacy of Asparaginase Encapsulated in Intact Erythrocytes," *Biochem. Pharmacol.* **34,** 257–261 (1985).
[56]. S.J. Updike, R.T. Wakarniya, and E.N. Lightfoot, "Asparaginase Entrapped in Red Blood Cells: Action and Survival,"*Science* **193,** 681–683 (1976)
[57]. R. Kravtozoff et al., "Erythrocytes as Carriers for L-Asparaginase: Methodological and Mouse In-Vivo Studies," *J. Pharm. Pharmacol.* **42,** 473–476 (1990).
[58]. D.A. Lewis and J. Desai, "The Use of Animal Models in the Encapsulation of Drugs in Erythrocytes," *Adv. Bio sci.* (series) **67,** 213–222 (1987).
[59]. E Venkatesh, C Aparna, K Umasankar, P Jayachandra Reddy, V Prabhakaran, *Int. J. Pharm. Sci. Rev. Res.*, **2013**, 23(2), 298-306
[60]. R Hudeca, B Lakatos, *Biochemical and Biophysical Research Communications*, **2004**, 325, 1172
[61]. D Raut, RS sakhare, KD Ketan, PD Halle. *IJRPC*, **2013**, 3(2), 198-207.
[62]. IJ Alvarez, *Biotechnology Biochemistry*, **1998**, 27, 139-143. [30] HO Alpar and WJ Irwin. *Adv. Biosci.*, **1987**, 67: 1–9.
[63]. A V Gothoskar, *Pharma. Tech. com*, **2004**, 140-158.
[64]. D Raut, RS sakhare, KD Ketan, PD Halle. *IJRPC*, **2013**, 3(2), 198-207.
[65]. Shashank shah, *International journal of pharma & bioscience*, **2011**, 2 (1), 394-406.
[66]. E Venkatesh, C Aparna, K Umasankar, P Jayachandra Reddy, V Prabhakaran, *Int. J. Pharm. Sci. Rev. Res.*, **2013**, 23(2), 298-306.
[67]. Pragya, V Rastogi, *International Journal of Pharmacy and Pharmaceutical Sciences*, **2012**, 4(3), 75-82.
[68].Orekhova et. al., Local prevention of thrombosis in animal arteries by means of magnetic targeting of aspirin-loaded red cells, 1990, 57, 611
[69]. M. Moorjani, A. Lejeune, C. Gicquaud, J. LAcroix, P. Poyet and R. C. Gaudereault, Nanoerythrosomes, A New Derivative of Erythrocyte Ghost II: Identification of the Mechanism of Action, Anticancer Res., 1996, 16 (5A), 2831–2836.
[70]. V. Jaitely et al. Resealed Erythrocytes: Drug Carrier Potentials and Biomedical Applications, Indian Drugs, 1996; 33:589– 594.
[71]. H. O. Alpar and D.A. Lewis. Therapeutic Efficacy of Asparaginase Encapsulated in Intact Erythrocytes, Biochem. Pharmacol. 1985; 34:257–261.
[72]. D. A. Lewis. Red Blood Cells for Drug Delivery, Pharm. J., 1984; 32: 384–385.
[73]. R. Baker. Entry of Ferritin into Human Red Cells during Hypotonic Haemolysis, Nature, 1967; 215: 424–425.
[74]. U. Sprandel. Towards Cellular Drug Targeting and Controlled Release of Drugs by Magnetic Fields, Adv. Biosci. (Series), 1987; 67: 243–250.
[75]. K. Kinosita and T.Y. Tsong. Survival of Sucrose-Loaded Erythrocytes in the Circulation, Nature, 1978; 272, 258–260.
[76]. H. G. Eichler et al. In Vivo Clearance of Antibody-Sensitized Human Drug Carrier Erythrocytes, Clin. Pharmacol. Ther, 1986; 40: 300– 303.
[77]. M. P. Summer. Recent Advances in Drug Delivery, Pharm. J., 1983; 230, 643–645.

[78]. H. C. Eichler et.al. In Vitro Drug Release from Human Carrier Erythrocyte AS Carrier System, Advance in Bioscience, 1987; 67:11-15.
[79]. J. R. DeLoach. Methods in Enzymology, Academic Press, New York, 1987; 235
[80]. Carmen Gutierrez Millan, Maria Luisa Sayalero Marinero, Aranzazu Zarzuelo Castaneda and Jose M. Lanao. Drug, enzyme and peptide delivery using erythrocytes as carriers, Journal of Controlled Release, 2004; 95(1): 27-49.
[81]. Mehrdad Hamidi, Adbolhossein Zarina, Mahshid Foroozesha and Soliman Mohammadi-Samania. Applications of carrier erythrocytes in delivery of biopharmaceuticals, Journal of Controlled Release, 2007; 118(2):145-1