Resealed Erythrocyte: An Approach to Targeted Drug Delivery (A Review)

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ABSTRACT

Application of erythrocytes, the most abundant cells of the human body with desirable physiologic and morphologic characteristics, in drug delivery has been exploited extensively. Among the various carriers used for targeting drugs to various body tissues, the cellular carriers meet several criteria desirables in clinical applications, among the most important being biocompatibility of carrier and its degradation products. Leucocytes, platelets, erythrocytes, Nano erythrocytes, hepatocytes, and fibroblasts etc. have been proposed as cellular carrier systems. Among these, the erythrocytes have been the most investigated and have found to possess greater potential in drug delivery. Biopharmaceuticals, therapeutically significant peptides and proteins, nucleic acid-based biological, antigens, anticancer drug and vaccines, are among the recently focused pharmaceuticals for being delivered using carrier erythrocytes. Erythrocytes, also known as red blood cells, and have been extensively 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.

Keywords: Resealed Erythrocytes, carrier, Isolation, Applications.

INTRODUCTION

The normal erythrocyte (normocyte) is a flexible, elastic, biconcave disc shaped structure with mean diameter 7.3 µm and thickness near 2.2 µm. The chemical constituent of red blood cells include water (63%), lipids (0.5%), glucose (0.8%), minerals (0.7%), non-hemoglobin (33.67%). The primary function of the erythrocyte is transport of oxygen and carbon dioxide. Erythrocytes are the most abundant cells in the human body (5.4 million cells/mm3 blood in a healthy male and 4.8 million cells/mm3 blood in a healthy female).

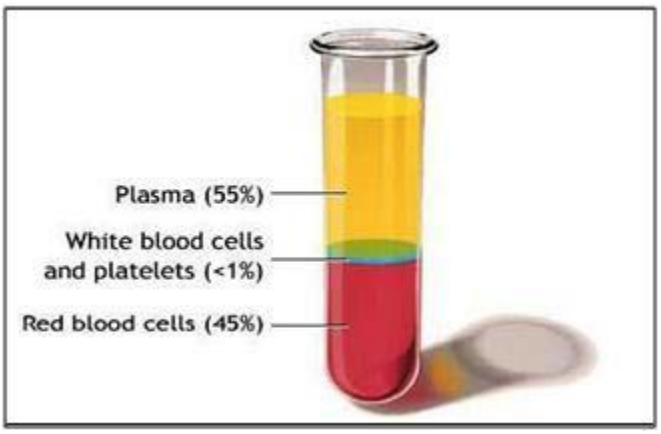

Figure 1: Composition of blood

Erythrocytes live only about 120 days because of wear and tear on their plasma membranes. The process of erythrocyte formation within the body is known as erythropoiesis. In a mature human being, erythrocytes are produced in red bone marrow under the regulation of a hemopoietic hormone called erythropoietin. [1]

ERYTHROCYTES

Red blood cells (also referred to as erythrocytes) are the most common type of blood cells and the vertebrate organism's principal means of delivering oxygen (O2) to the body tissues via the blood flow through the circulatory system. The cells develop in the bone marrow and circulate for about 100–120 days in the body before their components are recycled by macrophages. Each circulation takes about 20 seconds. Approximately a quarter of the cells in the human body are red blood cells. [2]

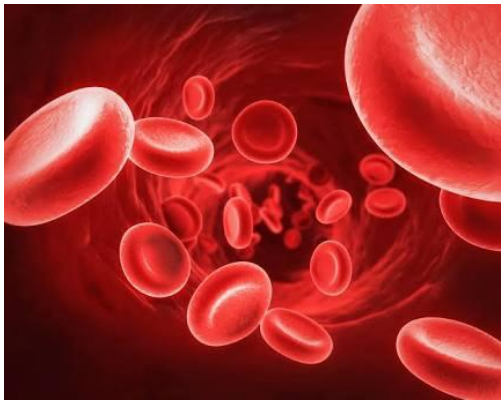

Fig 2: Erythrocyte

THE ERYTHROCYTES MEMBRANE

It contains about equal weight of proteins and lipids. The outer surface of membrane bears a net negative charge due largely to carboxylic groups of sialic acid. The isoelectric point is about pH 2 which is raised to pH4-5 after quantitative removal of the sialic acid with neural amidase. There is about 2.4×107 N-acetyl neuraminic acid residue per human erythrocyte. The negative surface charge prevents erythrocyte from coming into contact and maintains a sufficient distance between erythrocytes so that they cannot be readily agglutinated with IgG. [3]

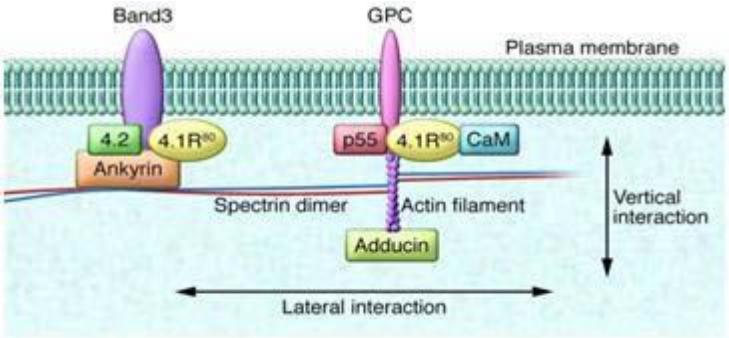

Figure 3: Basic structure of erythrocyte membrane

ADVANTAGES

- Biocompatible, particularly when autologous cells are used hence no possibility of triggered immune response.
- Biodegradability with no generation of toxic products.
- Considerable uniform size and shape of carrier.
- Relatively inert intracellular environment can be encapsulated in a small volume of cells.
- Isolation is easy and large amount of drug can be loaded.
- Prevention of degradation of the loaded drug from inactivation by endogenous chemical
- Entrapment of wide variety of chemicals can be possible.
- Entrapment of drug can be possible without chemical modification of the substance to be entrapped.
- Possible to maintain steady-state plasma concentration, decrease fluctuation in concentration.
- Protection of the organism against toxic effect of drug.
- Targeting to the organ of the RES.
- Ideal zero-order drug release kinetic.
- Prolong the systemic activity of drug by residing for a longer time in the body.11-20

DISADVANTAGES

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

ANATOMY PHYSIOLOGY AND COMPOSITION OF ERTHROCYTES

RBCs have shapes like biconcave discs with a diameter of 7.8 μm and thickness near 2.2 μm. Mature RBCs have a simple structure. It is also in elastic in nature. Their plasma membrane is both strong and flexible, which allows them to deform without rupturing as they squeeze through narrow capillaries. RBCs are highly specialized for their oxygen transport function, because their mature RBCs have no nucleus, all their internal space is available for oxygen transport. Each RBC contains about 280 million hemoglobin molecules. A hemoglobin 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 centers of the heme ring combine reversibly with one oxygen molecule, allowing each hemoglobin molecule to bind four oxygen molecules. RBCs include water (63%), lipids (0.5), glucose (0.8%), mineral (0.7%), non-hemoglobin protein (0.9%), methehemoglobin (0.5%), and hemoglobin (33.67%).[4]

ERYTHROCYTES CAN BE USED AS CARRIERS IN TWO WAYS

Targeting particular tissue/organ

For targeting, only the erythrocyte membrane is used. This is obtained by splitting the cell in hypotonic solution and after introducing the drug into the cells, allowing them to reseal into spheres. Such erythrocytes are called Red cell ghosts.

Continuous or prolonged release of drugs

Alternatively, erythrocytes can be used as a continuous or prolonged release system, which provide prolonged drug action. There are different methods for encapsulation of drugs within erythrocytes. They remain in the circulation for prolonged periods of time (up to 120 days) and release the entrapped drug at a slow and steady rate. [2]

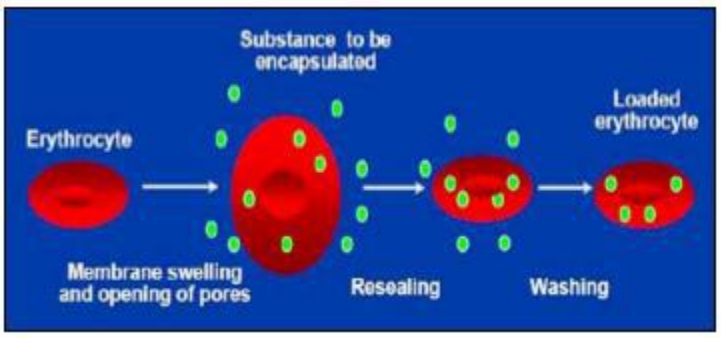

Fig 4: General mechanism of resealed erythrocytes

ISOLATION OF ERYTHROCYTES

- Blood is collected into heparin zed tubes by venipunture.
- Blood is withdrawn from cardiac/splenic puncture (in small animal) and through veins (in large animals) in a syringe containing a drop of anti-coagulant.
- The whole blood is centrifuged at 2500 rpm for 5 min. at 4 ±10C in a refrigerated centrifuge.
- The serum and Buffy coats are carefully removed and packed cells washed three Mtimes with phosphate buffer saline (pH=7.4).
- The washed erythrocytes are diluted with PBS and stored at 40C for as long as 48 h before use.
- 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. [4]

Table1; Various condition and centrifugal force used for isolation of erythrocytes

S.No.	Species	Washing Buffer	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

BASIC REQUIREMENT FOR ENCAPSULATION

- The compounds which are showing therapeutic action (5000-60,000 daltons) can be entraped or loaded in erythrocytes.
- Non- polar molecules may be entrapped in the form of salts. Example: tetracycline HCl salt can be appreciably entrapped in bovine Red Blood Erythrocytes.
- The compounds have either polar or non – polar molecules can have loaded in erythrocytes.
- Some molecules are hydrophobic in nature there are loaded by absorbing over the molecules.
- Charged molecules are stable long period of time when compared to uncharged molecule.
- The size of the drug molecule is significantly larger than B – galactosidase [5]

METHODS OF DRUG LOADING IN ERYTHROCYTES

1. Hypo-osmotic lysis

a) Dilution method

b) Preswelling method

c) Dialysis method

d) Osmotic lysis method

2. Membrane perturbation method

3. Electro encapsulation method

4. Endocytosis method
5. Lipid fusion method
6. Electric cell fusion method
7. Use of Red cell loader

1. **HYPO-OSMOTIC LYSIS METHOD:**

These are divided into four types are as follows-

a) Hypotonic Dilution

In this method, a volume of packed erythrocytes is diluted with 2–20 volumes of aqueous solution of a drug. The RBC'S are exposed to hypotonic solution (corresponding to 0.4% Nacl), the erythrocytic membrane ruptures permitting escape of cellular contents

and equilibrium is achieved with in one minute. The cells swell up to 1.6 times its original volume indicated by formation of pores of size 200 – 500 Ao. The length of time for which these pores remain open is not fixed. However, at 0oC the opening permits long enough to allow partial resealing of membrane. Increasing ionic strength to isotonicity and incubating the cells at 37oC causes the pores to close and restore osmotic properties of the RBC'S. This method is simplest and fastest yet the capsulation efficacy is very low i. e. 1 – 8%. Efficient for of low weight drugs.

Examples of encapsulated agents: β-glucosidase, asparaginase, arginase, salbutamol.

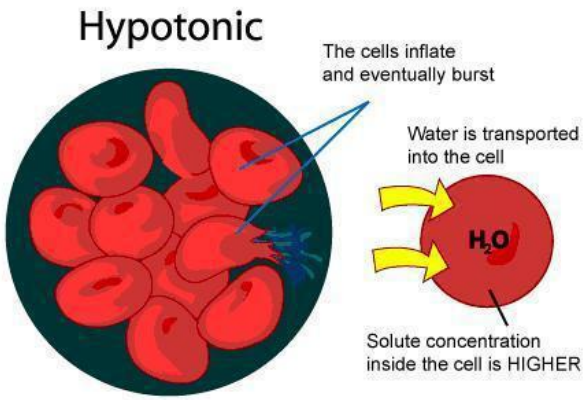

Fig 5: Hypotonic Dilution Method

b) Hypotonic preswell method

The method was investigated by Rechsteiner in 1975 and was modified by Jenner et al for-drug loading. This method was 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.

Examples of encapsulated agents: Propranolol, asparginase, methotrexate, isoniazid etc.

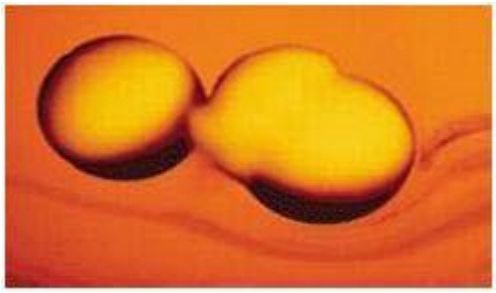

Fig 6: Hypotonic preswell Method

c) Hypotonic Dialysis

This method was first reported in 1959 by Klibansky and was used in 1977 by Deloach and Ihler and in 1989 by Gaudreault RC for loading enzymes and lipids. A desired Haematocrit is achieved by mixing washed erythrocyte suspension and phosphate buffer (pH 7.4) containing drug solution. This mixture is placed into dialysis bag and then both ends of the bag are tied with thread. An air bubble of nearly 25% of the internal volume is left in the tube. During dialysis bubble serves to blend the content. The tube is placed in a bottle containing 200ml of lysis buffer solution and placed on a mechanical rotator at 4oC for 2 hrs. The dialysis tube is then placed in 200 ml of resealing solution (isotonic PBS pH 7.4) at temperature 25 – 30oC for resealing. The loaded erythrocytes thus obtained are then washed with cold PBC at 4oC. The cells are finally resuspended in PBC.

Examples of encapsulated agents: Gentamicin, adriamycine, erythropoietin, furamycin A, IgG etc

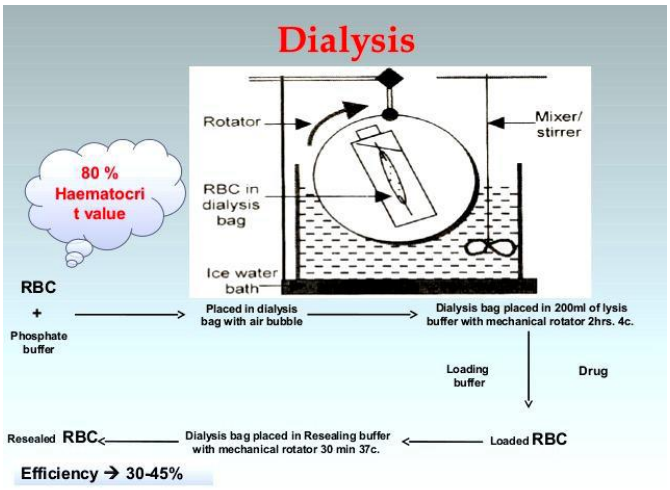

Fig 7: Hypotonic Dialysis Method

d) Isotonic osmotic lysis

This method, also known as the osmotic pulse method, involves isotonic hemolysis. Erythrocytes 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, polyethylene glycol, ammonium chloride and dimethyl sulfoxide (DMSO) have been used for isotonic hemolysis.

Examples of enc apsulated agents: Inositol hexaphosphate

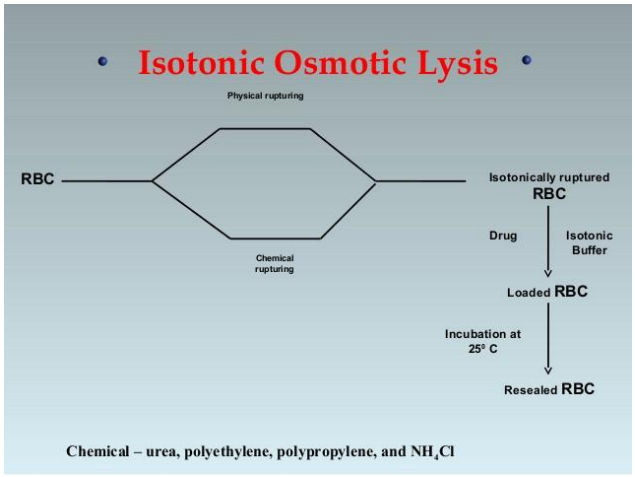

Fig 8: Isotonic Osmotic Lysis Method

2. 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 showed that the permeability of erythrocytic membrane increases upon exposure to polyene antibiotic such as amphotericin B. The method was used successfully by Kitao and Hattori to entrap the antineoplastic drug daunomycin in human and mouse erythrocytes in 1980. 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.

Examples of encapsulated agents: Daunomycin

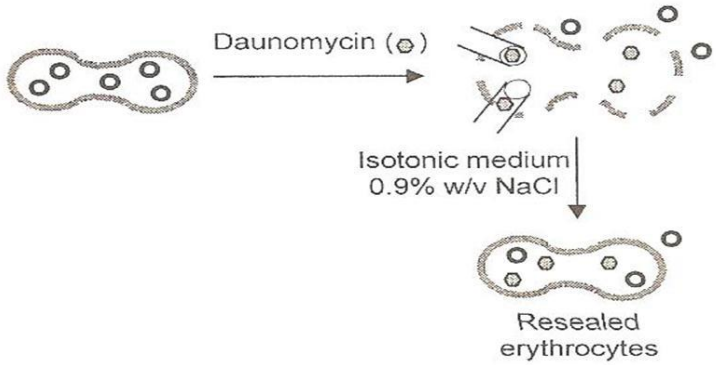

Fig 9: Drug loaded by perturbation method

3. ELECTRICAL BREAKDOWN METHOD/ ELECTOENCAPSULATION

When the membrane is polarized very rapidly (in nano to micro seconds) using voltage of about 2kV/ cm for 20µ sec electrical breakdown of a cell membrane is observed which leads to the formation of pores and entrapment of drugs. Electrical breakdown probably takes place in the lipoid regions or at the lipid protein junction in the membrane. Pores formed are stable and it is possible to control pore size. Subsequently the pores can be resealed by incubation at 37°C in osmotically balanced medium.

Examples of encapsulated agents: Primaquine and related 8–amino–quinolines, vinblastine, chlorpromazine and related phenothiazines, hydrocortisone, propranolol, vitamin A etc.

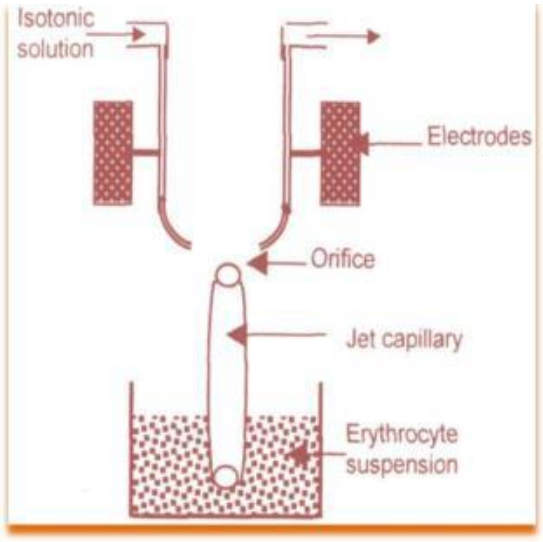

Fig 10: Drug loaded by electro encapsulation technique

4. ENDOCYTOSIS

This method was reported by Schrier (1987). Endocytosis involves the addition of one volume of washed packed erythrocytes to nine volumes of buffercontaining2.5 mm ATP, 2.5 mm $MgCl_2$ and 1mM $CaCl_2$, 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 370c for 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.

Examples of encapsulated agents. Hydrocortisone, propranolol, vitamin A Primaquine, vinblastine, chlorpromazine etc

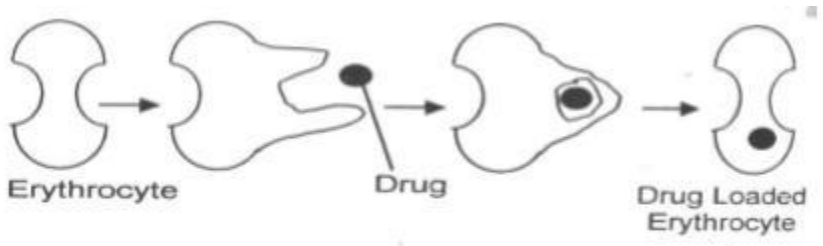

Fig 11: Drug loaded by Endocytosis

5. LOADING BY LIPID FUSION

In this method fused lipid vesicle containing bioactive molecule along with human erythrocytes leading to exchange of lipid entrapped drug molecule. This method provides very low encapsulation efficiency. Nicola and Gresonde fused lipid vesicle containing inositol hexaphosphate with human erythrocytes. The incorporated inositol hexaphosphate in erythrocytes provided a significant lowering of the oxygen affinity for hemoglobin in intact erythrocytes. Harrison et al reported resealing of tyrosine kinase into human erythrocytes by rapid freezing and thawing in liquid.

Examples of encapsulated agents: Inositol monophosphate.

6. 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.

USE OF RED CELL LOADER

Magnani and coworkers, 1998 developed a novel method for the entrapment of non-diffusible drugs into human erythrocytes. The equipment designed for this method was termed as "red cell loader". The method requires as little as 50ml of 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 hem filter 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. [6,7,8,9,10]

Fig.No.12 Use of Red Cell Loader

CONCLUSION

This review focuses on appropriateness of erythrocytes as biological carriers. It shows that some of the various latent biomedical applications of RBCs based drug delivery systems opening new perspectives to the vision of using our cells for salutary purposes. Thus, the resealed erythrocyte is the promising carrier for various drugs, therapeutic proteins, a vaccine for both targeting and delivery. However, the concept needs further optimization to become a custom drug delivery system. Most of the studies in this area are in the *in vitro* phase and the ongoing projects worldwide stay behind to step into preclinical and, then, clinical studies to prove the capabilities of this promising delivery system.

REFERENCES

1. Jain.S., Jain.N.K. "Resealed erythrocytes as drug carriers", Edited Jain N.K., Controlled and Novel Drug Delivery, CBS publishers, New Delhi, 2004; 256-281.

2. Solanki Dharmendra, Sharma Pragya, Motiwale, Mohit, Kushwah Lalit; resealled erythrocytes: a biocarriar for drug delivery; world journal of pharmacy and pharmaceutical sciences, Volume 6, Issue 4, 836-852

3. Gatkal S.H, Gaikwad N. M, Patil P. M, Chaudhari P.D**;** resealed erythrocyte: a novel drug delivery system; International Journal of Pharmaceutical Sciences Review and Research, Volume 12, Issue 2, January – February 2012; 42-50.

4. raut deepika b., sakhare ram s., dadge ketan k. and halle pd; resealed erythrocyte drug delivery: a review; international journal of research in pharmacy and chemistry, 2013, 3(2),198-207.

5. ramesh y, shaik. yasmin*, sravya silpa a, raj kumar k, gobinath m; resealed erythrocytes as drug carrier systems; international journal of pharmaceutics & drug analysis vol.4 issue 7, 2016; 343 – 350.

6. pati shubhangi jagannath, nalawade poonam shankar, mote ulka nathuram, haval prachi, gatade, prasad swati prakash; resealed erythrocytes: a novel approach for drug targeting; ejpmr, 2017,4(1), 263-272.

7. Lewis DA and Alpar HO; Therapeutic Possibilities of Drugs Encapsulated in Erythrocytes, Int. J. Pharm., 1984,1 -22, 137–146.

8. *Pitt E*, Johnson CM, Lewis DA, Jenner DA, Offord RE; Encapsulation of Drugs in Intact Erythrocytes: AnIntravenous Delivery System, Biochem. Pharmacol, 1983, 22: 359–368.

9. Nicolau C and Gersonde K; Incorporation of Inositol Hexaphosphate into Intact Red Blood Cells, I: Fusion of Effector- Containing Lipid Vesicles with Erythrocytes, Naturwissenschaften,1979, 66 (11), 563–566.

10. Schrier SL, Junga I, Johnson M; Energized Endocytosis in Human Erythrocyte Ghosts, J. Clin. Invest.,1975, 56 (1): 8–22.