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Review Article

RESEALED ERYTHROCYTE DRUG DELIVERY SYSTEM

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ABSTRACT

Erythrocytes, the most abundant cells in the human body, have potential carrier capabilities for the delivery of drugs. New drug delivery systems have been developed to overcome the limitations associated with the conventional drug delivery systems in order to improve the patient compliance and safety. They can be classified based on their size as (i) microcarriers for example, Liposomes, resealed, microspheres (ii) nanocarriers for example, niosomes, pharmacosome, aquasomes, solid lipid nanoparticles (SLN) (iii) variable carriers for example, dendrimers. Controlled Release drug delivery is one which delivers the drug at a predetermined rate, for locally or systemically, for a specified period of time. Patient comfort and compliance, Reduction in Health care cost, Patient variation are the advantages of controlled release drug delivery Systems, disadvantage are dumping, In-vitro In-vivo correlation, Increased potential for first pass effect. We are discusses mechanism of drug release such as- Immediate, modified, delayed & extended release. Red blood cells are the most common type of blood cells and the vertebrate organism's. Red blood cells (RBCs) have shapes like biconcave discs with a diameter of 7.8μm and thickness near 2.2μm. The several methods are present in this article Hypotonic Heamolysis, Hypotonic dilution, Isotonic Osmotic. various characterization parameters are discusses example- Shape & surface of morphology, Drug content, drug release. applications of resealed erythrocytes- In-vivo, Slow Drug Release, Drug targeting.

Key words: *Microspheres, Dendrimers, Isotonic osmosis, Control Release drug delivery.*

INTRODUCTION

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. Drug delivery is the method of formulation, technology or systems for transporting an active pharmaceutical ingredient to the target receptor at an organ in the body without any loss or compromise in the chemical integrity of the molecule and could be manipulated to effect the desired pharmacological action. Drug delivery technology plays around modification and improvement of various pharmacokinetic parameters such as drug release profile, absorption, distribution, and elimination, etc. The substances that are used to transport the drug to the target site were called as drug carriers and should aid the drug to prolong its in vivo actions, decrease metabolism, eliminates toxicity and with tailored pharmacokinetic parameters.[1] Resealed erythrocytes are prepared by the delivery of drugs and drug-loaded microspheres into the Erythrocytes, have been extensively studied for their potential carrier capabilities. Such drug-loaded carrier

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erythrocytes are prepared simply by collecting blood samples from the organism of interest, separating erythrocytes from plasma, entrapping drug in the erythrocytes, and resealing the resultant cellular carriers.

Controlled Release

Controlled Release drug delivery is one which delivers the drug at a predetermined rate, for locally or systemically, for a specified period of time. Controlled drug delivery systems can include the maintenance of drug levels within a desired range, the need for fewer administrations, optimal use of the drug in question, and increased patient compliance. While these advantages can be significant, the potential disadvantages cannot be ignored like the possible toxicity or non-biocompatibility of the materials used, undesirable by-products of degradation, any surgery required to implant or remove the system, the chance of patient discomfort from the delivery device, and the higher cost of controlled-release systems compared with traditional pharmaceutical formulations. The ideal drug delivery system should be inert, biocompatible, mechanically strong, comfortable for the patient, capable of achieving high drug loading, safe from accidental release, simple to administer and remove, and easy to fabricate and sterilize. The goal of many of the original controlled-release systems was to achieve a delivery profile that would yield a high blood level of the drug over a long period of time. With traditional drug delivery systems, the drug level in the blood follows the in which the level rises after each administration of the drug and then decreases until the next administration.

Controlled Released Dosage Form

The United States Pharmacopoeia (USP) defines the modified-release (MR) dosage form as "the one for which the drug release characteristics of time course and/or location are chosen to accomplish therapeutic or convenience objectives not offered by conventional dosage forms such as solutions, ointments, or promptly dissolving dosage forms". The terms "controlled release (CR)", "prolonged release", "sustained or slow release (SR)" and "long-acting (LA)" have been used synonymously with "extended release". Nearly all of the currently marketed monolithic oral ER dosage forms fall into one of the following two technologies:-

Hydrophilic, hydrophobic or inert matrix systems:- These consist of a rate controlling polymer matrix through which the drug is dissolved or dispersed.

Reservoir (coated) systems:- where drug containing core is enclosed within a polymer coating. Depending on the polymer used, two types of reservoir systems are considered.

- Simple diffusion/erosion systems where a drug-containing core is enclosed within hydrophilic and/or water-insoluble polymer coatings.
- Osmotic systems where the drug core is contained within a semi-permeable polymer membrane with a mechanical/laser drilled hole for drug delivery. Drug release is achieved by osmotic pressure generated within the tablet core.

Control Release Dosage Form Release Formulation Designs

Dissolution controlled release

- Encapsulation Dissolution control
- Seed or granule coated
- Micro encapsulation
- Matrix Dissolution control

Diffusion controlled release

- Reservoir type devices
- Diffusion and Dissolution controlled systems
- Ion exchange resins
- Osmotically controlled release

Advantages of Controlled Release Drug Delivery System

Therapeutic advantage:- Reduction in drug plasma level fluctuation, maintenance of a steady plasma level of the drug over a prolonged time period.

Reduction in adverse side effects and improvement in tolerability:- Drug plasma levels are maintained within a narrow

window with no sharp peaks and with AUC of plasma concentration Vs time curve comparable with total AUC from multiple dosing with immediate release dosage form.

Patient comfort and compliance:- Oral drug delivery is the most common and convenient for patient and a reduction in dosing frequency enhances compliance.

Reduction in Health care cost:- The total cost of therapy of the controlled release product could be comparable or lower than the immediate release product with reduction in side effects.

Patient variation:- The time period required for absorption of drug released from the dosage form may vary among individuals. Co-administration of other drugs, presence or absence of food and residence time in gastrointestinal tract is different among patients. This also gives rise to variation in clinical response among the patients.

Disadvantages of Controlled Release Drug Delivery System:-

Dose dumping:- Dose dumping can lead to fatalities in case of potent drugs, which have a narrow therapeutic index.

Less flexibility in accurate dose adjustment:-In conventional dosage forms, dose adjustments are much simpler.

Poor In-vitro In-vivo correlation:- In controlled release dosage form, the rate of drug release is deliberately reduced to achieve drug release possibly over a large region of gastrointestinal tract.

Increased potential for first pass clearance: Hepatic clearance is a saturable process. After oral dosing, the drug reaches the liver via portal vein.

Properties Influencing the Controlled Release Dosage Form

Most important factor is properties of the drug that are as follows.

Physicochemical properties:-

Aqueous solubility and pKa:- Absorption of poorly soluble drugs is often dissolution rate-limited. Such drugs do not require any further control over their dissolution rate and thus may not seem to be good candidates for oral controlled release formulations.

Partition coefficient :- Drugs that are very lipid soluble or very water-soluble i.e., extremes in partition coefficient, will demonstrate either low flux into the tissues or rapid flux followed by accumulation in tissues.

Stability of the drug:- Since most oral controlled release systems are designed to release their contents over much of the length of GI tract, drugs that are unstable in the environment of the intestine might be difficult to formulate into prolonged release system.

Size of the dose:- For drugs with an elimination half-life of less than **2 hours** as well as those administered in large dosages, a controlled release dosage form may need to carry a prohibitively large quantity of drug.

Molecular size and diffusivity:- In addition to diffusion through a variety of biological membranes, drugs in many sustained release systems must diffuse through a rate controlling membrane or matrix. The ability of drug to pass through membranes, its so called diffusivity, is a function of its molecular size (or molecular weight).

Biological properties:-

Absorption:- Slowly absorbed drugs or the drugs absorbed with n rate are **poor** candidates for a controlled release system.

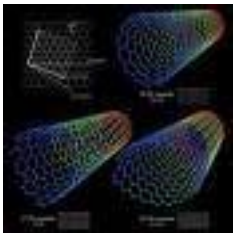
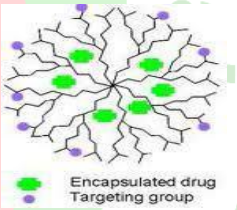
Metabolism:- Drug metabolism can result in either inactivation of an active drug or conversion of an inactive drug to an active metabolite.

Elimination or Biological half-life:- The rate of elimination of drug is described quantitatively by its biological half-life.

Safety considerations and Side effects:- For certain drugs the incidence of side effects is

believed to be a function of plasma concentration.

Table.1:Some Examples of Controlled Release Dosage Forms

S.N.	Nanocarriers	Discription	Images
1.	**Nano tube**	They are hollow cylinder made of carbon, atoms which can be filled and sealed for potential drug delivery. Help to identify DNA changes associated with cancer cells.	
2.	**Nano cells**	Nano shells are hollow silica spheres covered with gold. Has potential for targeting cancerous drug.	
3.	**Quantum dots**	These are tiny crystals that glow when these are stimulated by ultraviolet light.	
4.	**Dendrimers**	Dendrimers are new class of macromolecules which have a symmetric core and form the 3-D spherical structure.	

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. They take up oxygen in the lungs or gills and release it while squeezing through the body's capillaries. These cells' cytoplasm is rich in hemoglobin, an iron-containing bimolecule that can bind oxygen and is responsible for the blood's red colour. 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. Red blood cells (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. Each RBC contains about 280 million hemoglobin molecules. A globin is a protein present in hemoglobin molecules, consist of four polypeptide chains; to each of the four chain, a non-protein pigment called a heme, is bound to it. RBCs include water (63%), lipids (0.5), glucose (0.8%), mineral (0.7%), non-hemoglobin protein (0.9%), meth hemoglobin (0.5%), and hemoglobin (33.67%).[3]

Composition of Erythrocytes

- Blood contains about 55% of fluid portion (plasma) 45% of corpuscles or formed elements.
- Normal blood cells have extensile, elastic, biconcave and non-nucleated configuration with a diameter ranging from 6-9 μ and the thickness is nearly 1-2 μ.
- Erythrocytes have a solid content of about 35% most of which is Hb and rest 65% being water.

Electrolyte composition of Erythrocytes:

- The concentration of K+ is more in erythrocytes and Na+ in plasma.
- The osmotic pressure of the interior of the erythrocytes is equal to that of the plasma and termed as isotonic (0.9% NaCl or normal physiological saline.)
- Changes in the osmotic pressure of the medium surrounding the red blood cells changes the morphology of the cells.
- If the medium is Hypotonic water diffuses into the cells and they get swelled and eventually lose all their hemoglobin content and may burst.

Source 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.

Isolation of Erythrocytes

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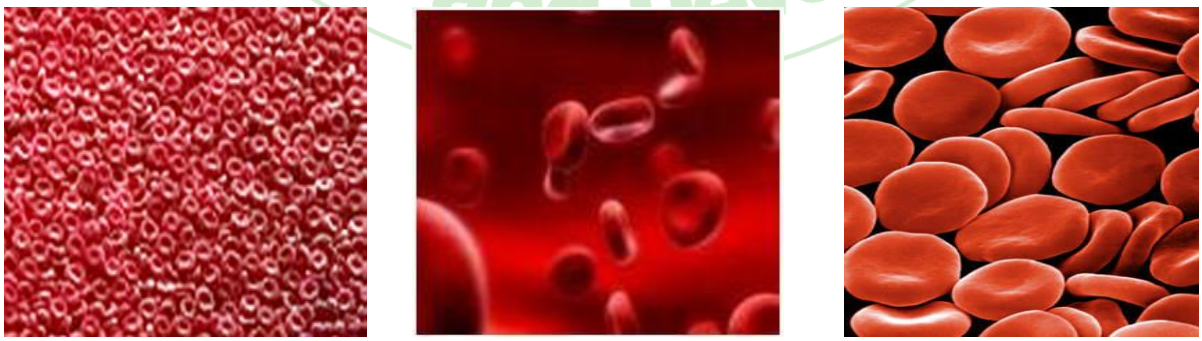

Separation process **Isolated erythrocytes** **Resealed Erythrocytes**

Fig. 1: Isolated RBCs

Resealed Erythrocytes: Such drug-loaded carrier erythrocytes are prepared simply by collecting blood samples from the organism of interest, separating erythrocytes from plasma, entrapping drug in the erythrocytes, and resealing the resultant cellular carriers. Hence, these carriers are called resealed erythrocytes.

Advantages of Resealed Erythrocytes as Drug Carriers:

The resealed erythrocytes should have the following advantages.

- Their biocompatibility, particularly when autologous cells are used, hence no possibility of triggered immune response 13.
- Their biodegradability with no generation of toxic products 14.
- The considerably uniform size and shape of the carrier 15.
- Relatively inert intracellular environment 16

Disadvantage of Resealed Erythrocytes:

- The rapid leakage of certain encapsulated substances from the loaded erythrocytes.
- Several molecules may alter the physiology of the erythrocyte.
- They have a limited potential as carrier to non-phagocyte target tissue.
- Possibility of clumping of cells and dose dumping may be there.

Mechanism of Drug Release:-

- **Immediate release-** drug is released immediately after administration.
- **Modified release** – drug release only occurs some time after the administration or for a prolonged period of time or to a specific target in the body. Modified release systems can be further classified as:
- **Delayed release:** drug is released only atsome point after the initial administration.
- **Extended release:** prolongs the release to reduce dosing frequency

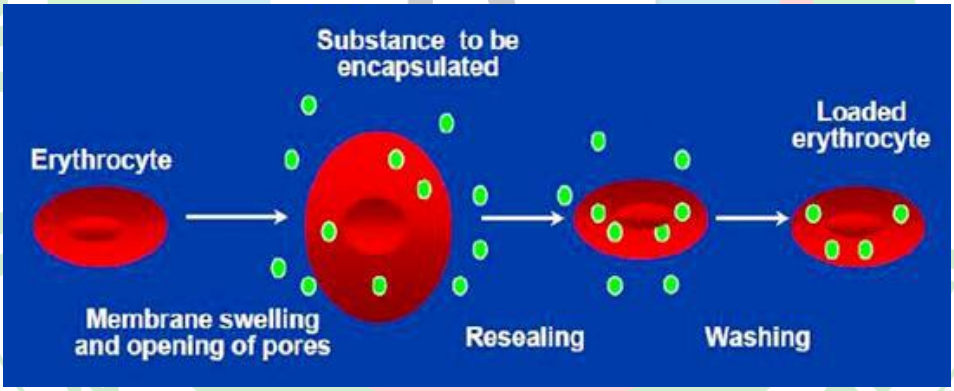

Fig. 2: General Mechanism Of Resealing Of Erythrocyte

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]

Targeted drug delivery

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

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:

Various Characterization Parameters and Their Determination Methods For Resealed Erythrocytes:

Table.1: Physical Parameter

Parameter	Method /Instrument Used
Shape and surface morphology	Transmission electron microscopy, scanning electron microscopy,
Vesicle size and size distribution	Transmission electron microscopy, optical microscopy Diffusion cell, dialysis
Drug release	Deproteinization of cell membrane followed

Table.2: Cellular Characterization

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
Erythrocyte sedimentation rate	ESR methods

Table.2: Biological Characterization

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

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.

In-vivo applications
Slow Drug Release: Erythrocytes have been used as circulating depots for the sustained delivery of antineoplastics 68, antiparasitics, veterinary, anti-amoebics, vitamins, steroids, antibiotics and cardiovascular drugs 69.

Drug targeting
Preferably to exhibit maximal therapeutic index with minimum adverse effects drug delivery should be site specific and target oriented. Resealed erythrocytes not only act as drug carriers and also as targeting tool.

Targeting RES organs
MPS/RES are targeted by surface modified erythrocytes. Since the change in the membrane is renowned by macrophages. The various approaches used include: Surface alteration with antibodie, glutaraldehyde, sulfhydryl, surface chemical cross-linking, carbohydrates such as sialic acid.

Removal of toxic agents
Reserve of cyanide intoxication with murine carrier erythrocyte containing bovine rhodanese and sodium thiosulfate.

Delivery of therapeutic agents
Erythrocytes have been used for release of so many therapeutic agents from several curative groups, both registered and in advance ones. As a basic approach in this review, all studied drugs will be classified into two main groups named pharmaceuticals and biopharmaceuticals.

Anticancer agents
Usually loading anticancer drugs into carriers reins their toxicity to the body in addition of improving their delivery to tumors relies via several mechanisms, including both specific and less specific (i.e., passive targeting for example enhanced permeation and retention effect, EPR, typical of solid tumors).

Anti-infective agents
For the delivery of three main anti-infective groups including anti-parasitic, antibiotic, antifungal and anti babesiosis (in veterinary) agents carrier erythrocytes have been used.

Corticosteroids
Prolonged delivery of dexamethasone which has been tested in vivo in rabbits and humans is the most developed application of carrier erythrocyte..

Cardiovascular drugs
Using a hypotonic preswelling dilution method, have shown that human erythrocytes loaded by enalaprilat release the drug *in vitro* according tozero-order kinetics.

Iron chelators
For treatment of iron over accumulation in the thalasemic patients and other forms of anemia that require regular transfusions for that carrier erythrocytes, encapsulated with desferrioxamin (DF), have been studied widely As discussed above, the RES is the main site of destruction of old erythrocytes and, consequently, of iron over-accumulation in these patients.

CONCLUSION
Now a day's there are numerous applications have been proposed for the use of resealed erythrocytes as carrier for drugs, enzyme replacement therapy etc. Until other carrier systems come of age, resealed erythrocytes technology will remain an active field for the further research. The use of resealed erythrocytes shows potential for a safe and effective delivery of various bioactive molecules for effective targeting. However, the concept needs further optimization to be converted into a regular drug delivery system. The coming years represent a significant time in this field as commercial applications are explored. In coming future, erythrocytes based delivery system with their capability to afford

controlled and site specific drug delivery have been developed for disease management. Erythrocyte carriers are **"nano device in field of nanotechnology**".

Main suggestion for future study is that by carrier through we can transplant steroids and hormones to the targeting site. So we can decrease many side effects. By resealed erythrocyte we can improvise drug targeting area and reduces so many side effect. For the present, it is concluded that erythrocyte carriers are **"golden eggs in novel drug delivery systems"** considering their tremendous potential.

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