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VALUE ADD

In-vitro drug release methods for liposomal drug delivery systems

Aditya Marfatia, Director, Electrolab and **Dr Neelam Sayed**, Application Scientist, Electrolab, provide an overview of the current compendial and non-compendial methods used for in-vitro release testing of liposomes

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Neelam Sayed

NANOPARTICULATE systems have emerged as prevalent drug delivery systems over the past few decades. These delivery systems have been extensively used to improve bioavailability, prolong pharmacological effects, achieve targeted drug delivery, as well as reduced side effects. The increasing interest in nanotechnology-based drug delivery systems has been a key factor in the design and development of numerous novel dosage forms and complex delivery therapies such as liposomes, nanoemulsions, polymeric nanoparticles, nanofibers and dendrimers etc to treat a variety of diseases.

Liposomes are one of the most tested and versatile systems among all the lipid-based nanotechnologies for drug delivery. They are composed of lipid bilayers enclosing an aqueous core in the centre. Drugs can be either loaded into the aqueous core or bound to the lipids within the membrane. The sizes of liposomes range from 1 µm for multilamellar vesicles to 4-8 nm for very small particles. Since the liposomes in clinical use have an average size of 50 to 200 nm, liposomes are commonly referred to as nanoparticles. Liposomes have been successfully used in the delivery of anti-cancer agents and liposomal formulations of Doxorubicin (Myocet, Doxil) and Daunorubicin (DaunoXome) have been approved by the FDA for clinical use.

Although liposomal drug delivery systems are promising for the treatment of a variety of human diseases, but an unanticipated change in product quality or performance of such systems may result in toxicity and/or change in-vivo efficacy, hence it is essential to develop suitable in-vitro dissolution/release testing methods to ensure product quality and performance and to assist in product development.

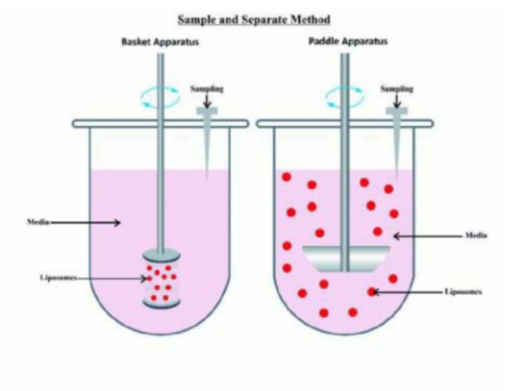
Despite the great strides in design and development of liposomes, no compendial or regulatory standards exist for in-vitro release testing. Although there have been attempts to use the existing USP apparatus for in-vitro drug assessment, the setups were designed primarily for oral and transdermal products and as such pose many challenges during a release study. Hence, several in-vitro release methods, both compendial and non-compendial, have been utilised and reported. Certainly, the area of in-vitro testing for liposomal drug delivery systems lags behind the advances realised in drug product development.

The present review provides an overview of the current compendial and non-compendial methods used for in-vitro release testing of liposomes. These methods can be broadly divided into three categories such as Sample and Separate (SS), Continuous Flow (CF) method and Dialysis Method (DM). More recently, apparatus that combine the principles of either the SS and DM or CF and DM have also been reported. All these methods along with adaptations, additional considerations and limitations are discussed below.

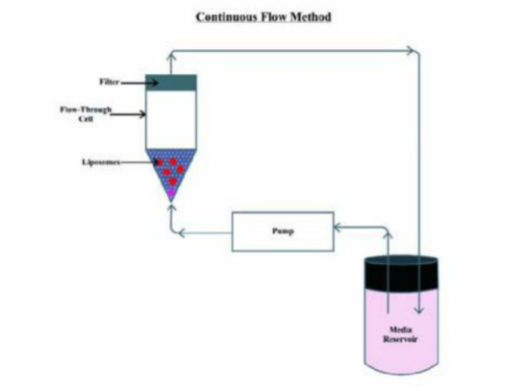
Drug release is monitored by physically separating the liposomes from the release

Fig.1: Schematic representation of compendial and non-compendial methods used for in-vitro release testing of Liposomal Drug Delivery Systems

1. SAMPLE AND SEPARATE METHOD



2. CONTINUOUS FLOW METHOD:



media, followed by analysis of the former or the latter. There are several adaptations to the SS method, with differences noted in set-up, container size, mode of agitation and sampling techniques. Aggregation of nanoparticles during in-vitro release appears to be a key concern. Additionally, clogging of filters during sampling and adsorption of drug to the filter has also been reported.

The availability of automated equipment has simplified routine sampling and media replacement with the CF method in open (nonrecirculating media) and closed (recirculating media) loop systems. However, the CF methods suffer from few limitations that include instrument costs, difficulty in setup, filter clogging, adsorption to the filter etc.

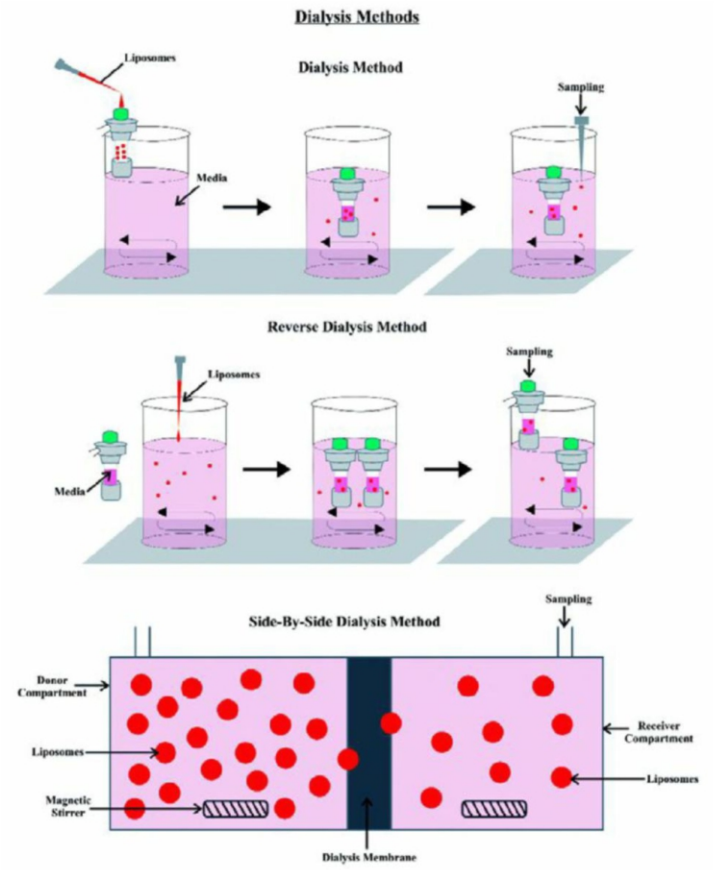
As with the other methods, several adaptations of the DM have been reported in literature with key differences in set-up, container size and molecular weight cut-off (MWCO) of the membrane. The basic premise of the DM is that drug from the dosage form diffuses rapidly from one compartment, through the membrane and enters the second compartment from where it is sampled for analysis. Thus, membranes with a sufficiently high membrane MWCO are often selected for in-vitro release studies so that drug transport is not a limiting factor. If incorrectly sealed, leakage of media and dosage form may occur from both ends of the dialysis bag set-up. Another factor to be considered is that the DM cannot be used with drugs that bind to the dialysis membrane.

Apart from the commonly reported SS, CF and DM methods; another alternate approach has been recently utilised to monitor drug release from novel dosage forms using the Rotating Bottle Apparatus (RBA). In this apparatus, bottles are clipped into a rotating shaft which is immersed in a water bath and the shaft is rotated at a desired speed. Dissolution aliquots are collected at specified time points and analysed using suitable analytical technique. In 1970s, Professor Beckett and many workers used RBA to assess the dissolution profiles of extended-release products especially those in the pellet form. Since then, RBA has been reported to be used in drug release testing of tablets and capsules, suppository, microspheres, nanoparticulates, implants, stents and also to evaluate milk-clotting activity.

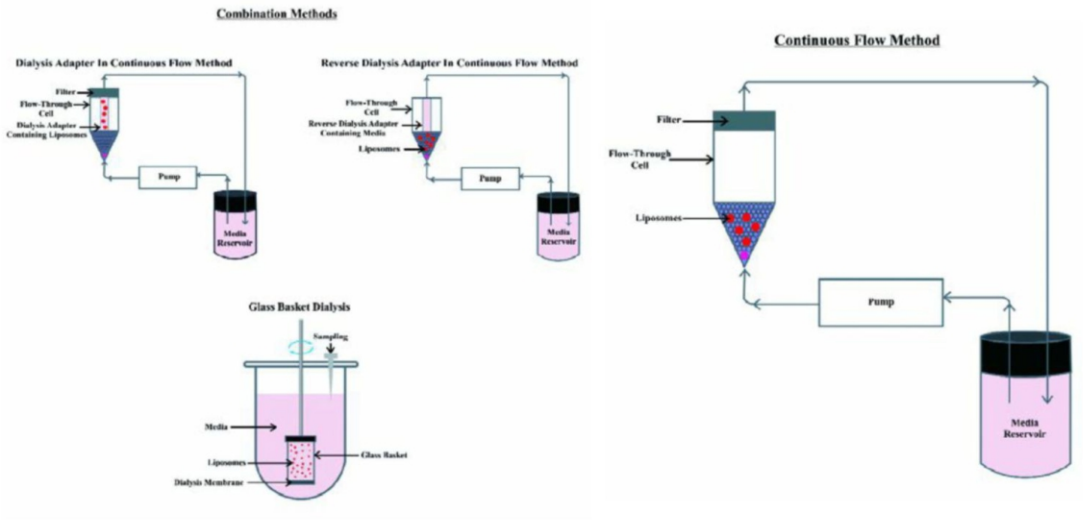
Recently, the application of RBA was extended to compare the drug release profiles of Pegylated Doxorubicin Hydrochloride Liposome (PEGADRIA) and commercially available reference product Doxil. Doxil is a formulation that encapsulates Doxorubicin in an aqueous compartment of liposome. Doxorubicin is one of the most widely used chemotherapeutic agents and is effective against variety of tumours, leukemias, sarcomas and breast cancer. The in-vitro release of PEGADRIA and Doxil were measured in Phosphate-Buffered Saline (PBS), pH 6.5, using Electrolab Rotating Bottle Apparatus (ERB-40A). In order to evaluate the effect of temperature on the lipid bilayer, the release experiments were carried out at 37°C and 47°C.

Both the PEGADRIA and Doxil showed similar in-vitro release profiles. The results confirmed that Doxorubicin was slowly released from liposomes during in-vitro incubation in PBS at 37°C but as expected it

3. DIALYSIS METHOD:



4. COMBINATION METHODS



was found to be temperature dependent. The temperature dependent drug release patterns of Doxorubicin from PEGADRIA and Doxil are depicted in *Figure 2*. In both cases, the release of Doxorubicin was about 90 per cent at 47°C after 24 hours.

The application of ERB-40A to evaluate the in-vitro release of PEGADRIA and Doxil from 50 per cent human plasma has also been discussed in this research article.

RBA presents important advantages over other apparatuses by providing a sound hydrodynamic system i.e. the dosage form moves freely through the dissolution medium as the bottle is rotated. This is in contrast to the movement of media relative to the dosage form in USP apparatus 1 and 2, wherein various portions of the bulk medium move at different rates. In addition, RBA enables small volume dissolution environment, accelerated drug release studies, facilitates pH profiling, prevents evaporative loss and offers higher reproducibility.

ERB-Wu, a 21 CFR part 11 compliant system offers easily interchangeable rotating shaft/rack that holds test bottles of 10 mL, 100 mL, 200 mL and 250 mL capacity. ERB-Wu water bath facilitates better temperature control and heat transfer for short duration as well as long duration (several months) in-vitro release studies. The long duration in-vitro release studies can be carried out uninterrupted, without cleaning the water bath for several months by integrating ERB-Wu with ClearView, a proven ultraviolet technology that significantly reduces frequent water bath cleaning. In-vitro release methods can be developed using ERB-Wu by optimising various test parameters like rotational speed, temperature, media volume, test duration ie. short duration and long duration test with or without replenishment. System can be validated for various parameters like rotational speed, temperature and timer at regular intervals as per laboratory requirements.

For novel dosage forms like liposomes where no regulatory or compendial standards exist, in-vitro drug release assessment assumes greater significance in serving as an indicator of product quality and performance. As described, a plethora of methods have been used; each with their advantages and limitations with respect to ease of set-up, sampling and rapid buffer replacement. Ideally, an in-vitro release method should be discriminatory and be able to simulate in-vivo conditions, release mechanisms and enable the establishment of an IVIVC.

FIG.2: IN-VITRO RELEASE STUDY OF PEGADRIA AND DOXIL AT VARIOUS TEMPERATURES

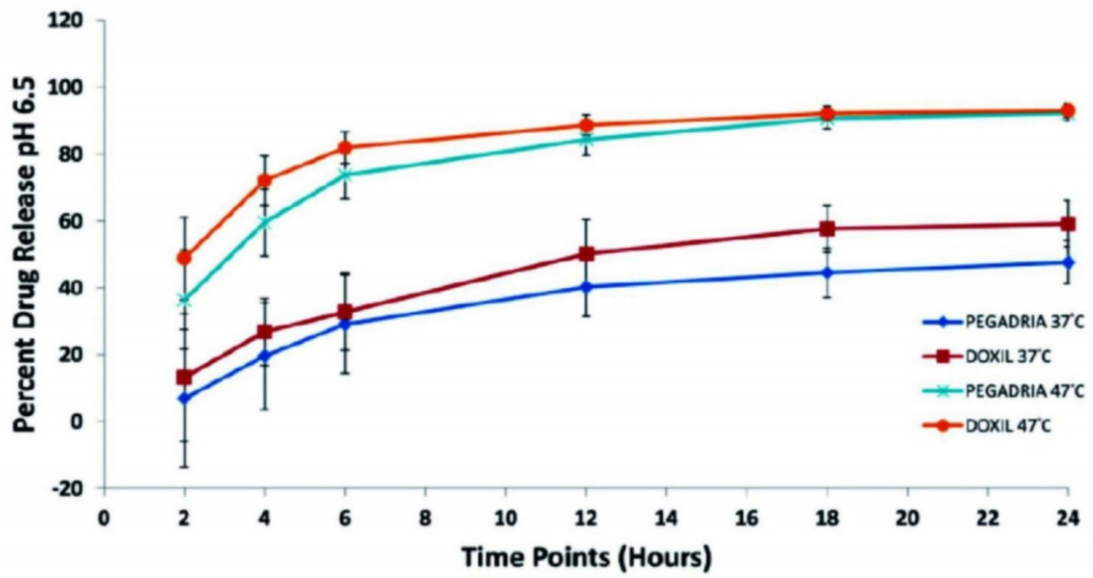
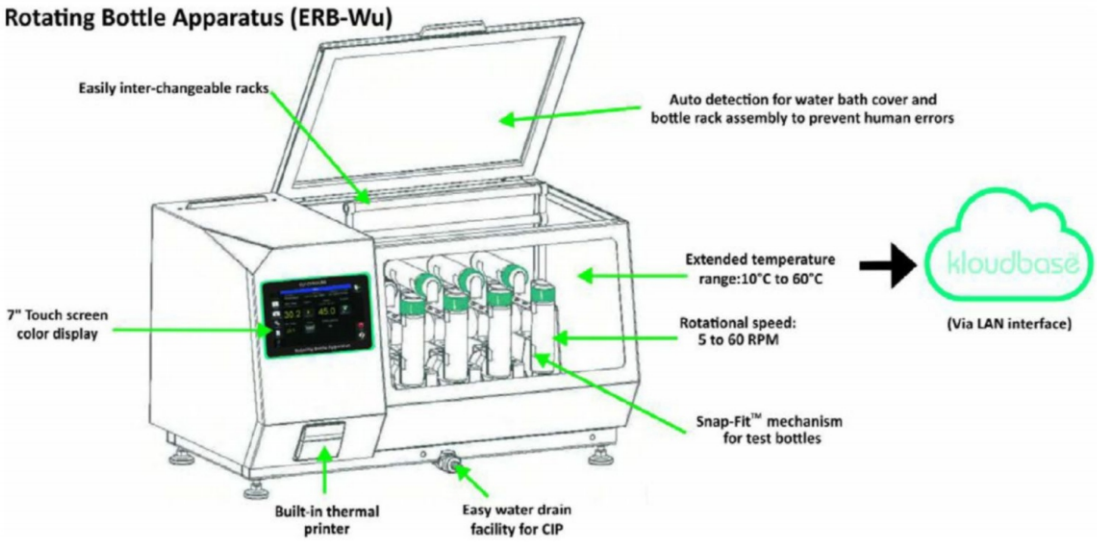


FIG. 3: ELECTROLAB ROTATING BOTTLE APPARATUS (ERB-WU):



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