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Physical Stability of Rivaroxaban Loaded Liposomes Coated with Low Molecular Weight Chitosan

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Abstract: ***The purpose of this study is to evaluate the improvement in physical stability of Chitosan (CS) coated liposomes. Small unilamellar classic liposomes (RIV-CLs) and flexible liposomes (RIV-FLs) loaded with rivaroxaban (RIV) were coated with low molecular weight chitosan (LMW CS) by simply mixing the liposomal suspension with the acidic polymer solutions of varying strengths. Changes in particle size and zeta potential for CS coated liposomes confirmed the existence of a thick polymer layer on the surface of liposomes which was also manifested from the TEM images. The physical stability of CS coated liposomes was evaluated by measuring the change in particle size, zeta potential, in vitro drug release and the retention of entrapped contents after periods of storage.***

Keywords: Rivaroxaban, Liposomes, Chitosan, In vitro, Stability

I. INTRODUCTION

Oral delivery of liposomes also has a long history and can be traced back to the late 1970s. It is interesting to note that the initial use of oral liposomes involved the delivery of insulin, underlining the continual interest in the area of oral drug delivery. In order to protect liposomes from the harsh gastrointestinal environment, the treatment of liposomal surfaces with polymer layers such as enteric polymers, proteins and chitosans was a workable approach (Barea et al., 2010; Barea et al., 2012; Hosny et al., 2013).

Enteric coatings were proven to protect liposomal vesicles from being disrupted in the gastric environment thereby enhancing absorption as more intact vesicles are delivered to the small intestine. Liposomes coated with Eudragit L100 improved the oral bioavailability of alendronate sodium by more than 10 folds as compared with the marketed tablets (Hosny et al., 2013). However, during certain trials, layers of coating with enteric polymers such as Eudragit S100 cannot overcome the strong damaging effect of bile salts (Barea et al., 2010). Consequently, a model comprising liposomes-in-microspheres delivery systems enclosing chitosan-coated liposomes within Eudragit S100 microspheres was proved to be highly competent to survive the damage by bile salts (Barea et al., 2012).

CS mediated mucoadhesion imparts liposomes a prolonged GIT residence, offering a prolonged contact of liposomes and their payloads with intestinal epithelia and, hence improving their oral absorption. Improving mucoadhesion is established via coating with polymers or modulating surface charges. For instance, liposomes bearing a positive charge have not only mucoadhesion but also higher resistance to enzymatic destruction (Takeuchi et al., 2003), and consequently, improve oral bioavailability of the active materials in their payloads (Hashimoto and Kawada, 1979).

Polymer coating of liposomes seems to be one of the most promising approaches to achieve mucoadhesion (Han et al., 2012; Thirawong et al., 2008; Thongborisute et al., 2006). CS represents one of the most attractive coating polymers for oral liposomes because of its low toxicity, biocompatibility, biodegradability and mucoadhesion.

Liposomes are first prepared for the preparation of CS-coated liposomal formulations using various well-known methods such as thin-lipid film hydration, reverse-phase evaporation and organic phase injection (Patil and Jadhav, 2014). The acidic CS solution is applied dropwise to the vesicles under magnetic stirring after core liposomes have been prepared. The mixture solution is then mixed at room temperature for about 1 hour with gentle shaking and then incubated at 4 ° C (Berginc et al., 2014). It should also be noted that some researchers proposed that CS coated liposomes could be prepared by adding pure liposome suspension to the CS solution (Karewicz et al., 2013; Zhao et al., 2015; Zhuang et al., 2010). Nevertheless, there are no significant differences between CS-Lip characteristics developed by applying liposomal suspension to the CS solution and those prepared by CS in addition to the liposomes.

Most CS coated liposomal formulations are developed on the surface of liposomal bilayers and-NH3 + groups of CS chains through electrostatic interactions between phospholipid polar head groups. Hydrophobic interactions between phospholipid apolar tails and

CS backbone, on the other hand, can facilitate the integration of the polymer chains into the liposomal bilayer without loss of structural liposome organization.

Previous studies have shown that coating with CS can have both positive and negative effects on EE%. With regard to the risk of drug ionization at acid pH values, EE% change may be due to electrostatic interaction between the drug carriers (Prabhu et al., 2012; Vural et al., 2011; Zaru et al., 2009). Conversely, in addition to competing for interaction with liposomal bilayer, the mutual repulsion between CS and positively charged drugs may decrease the EE drug (Gonzalez-Rodriguez et al., 2007; Mehanna et al., 2010). RIV is used in the treatment of pulmonary embolism (PE) and deep vein thrombosis (DVT). Due to unsatisfactory therapeutic effectiveness and toxic effect, conventional anticoagulant medications such as vitamin K, unfractionated heparin and warfarin are not commonly used in the context of clinical practice.

RIV is an effective factor Xa (FXa) inhibitor. Of activity it does not need a cofactor and directly inhibits free action of FXa and prothrombinase. Such advantages ascribed to the broad application of RIV in clinical medicine. Nevertheless, RIV's low solubility in aqueous media indirectly contributes to a discrepancy in the drug's bioavailability between fasted and fed condition. The problem of low aqueous solubility of BCSII products, such as RIV, has been solved using various techniques such as formulation in lipidic carriers such as liposomes.

CS-coated liposomes have a high capacity for mucoadhesion, increased carrier permeability, and sterically stabilized properties. Such promising carriers can provide a solution for fasted RIV's impaired oral bioavailability and can be used in liquid form to treat pediatric patients.

II. EXPERIMENTAL

A. Materials

Rivaroxaban (RIV) was supplied by Al Andalous Pharmaceutical Industries Co., Cairo, Egypt. Phospholipid (PL), LIPOID S PC, injection grade phosphatidylcholine from soybean was a generous gift from Lipoid GmbH, Germany. Chitosan (CS), Low molecular weight (mol wt 50,000-190,000 Da, based on viscosity), degree of deacylation ≥ 75 % and Cholesterol (CH), from sheep wool, sigma grade, were purchased from Sigma-Aldrich CHEMIE GmbH, Saint Louis. Tween 80 (T 80) and Dimethyl Sulfoxide (DMSO), Molecular weight 78.13, minimum assay 99% were supplied by ALPHA CHEMIKA Co., Mumbai, India. Span 80 (S 80), was supplied by Oxford Lab Chem Co., India. Methanol Analar was obtained from SD Fine-Chem limited, Mumbai, India. Chloroform was supplied by Honeywell Specialty Chemicals Seelze GmbH, Seelze, Germany. Glacial acetic acid was obtained from RESEARCH LAB FINE CHEM INDUSTRIES, Mumbai, India. All reagents were analytical grade and used without further purification.

III. METHODOLOGY

A. UV Spectrophotometric Determination of RIV

A validated UV spectrophotometric method developed by Sekaran et al. for the quantification of RIV in bulk was employed. According to this method, RIV was determined at 270 nm after dissolution in DMSO (Sekaran et al., 2013). For this purpose, a stock standard solution containing 1 mg mL-1 of RIV was prepared in DMSO. Working standard solution equivalent to 100 µg mL-1 of RIV was prepared by appropriate dilution of stock solution with the same solvent.

Aliquots of 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mL of 100 µg mL-1 RIV working standard solution were accurately transferred into a series of 5 mL calibrated flask and made up to the mark with DMSO. This results in standard solutions with the following concentrations: 2, 4, 8, 12, 16 & 20 µg ml-1 respectively.

The UV absorbance of the standard solutions was determined at 270 nm versus DMSO blank employing UV spectrophotometer SHIMADZU UV-1800, Tokyo, Japan. Calibration curve was constructed by plotting the absorbance versus concentration of RIV. The unknown concentration of the samples was estimated from the calibration curve or using the formula derived employing Beer's law.

Placebo blank solution was prepared from a mixture of the excipients employed in the formulations. PL (60 mg), CH (20 mg) and CS (5 mg) were dissolved in 5 ml of 0.1% glacial acetic acid, T 80 (15 mg) and S 80 (15 mg) were accurately weighed and mixed well. The mixtures were then transferred to a 50 ml calibrated flask and 30 mL DMSO was added to the flask and the content was shaken thoroughly for 20 minutes. The final volume was adjusted to the 50 ml mark with fresh DMSO, thoroughly mixed then filtered using No. 1 Whatman filter paper. One mL of the filtrate (placebo blank solution) was diluted to 5 mL with DMSO. This solution was employed as the blank for determination of RXV in the formulations.

B. Formulation of RIV liposomes

1) Preparation of RIV Loaded classic Liposomes (RIV-CLs) and flexible Liposomes (RIV-FLs): Many conventional methods for preparation of multilamellar liposomal vesicles have been described. In this study, the thin film hydration method has been employed. The composition of the proposed formulations is illustrated in table (1). Briefly, the PL, CH, T80/ S80 along with RIV were accurately weighed and dissolved in a mixture of methanol and chloroform in 1:1 ratio. The resultant solution was transferred to the clean, oven dried round bottom flask of a rotary evaporator. The temperature of the water bath was adjusted to 50° C. The resulting thin film was kept under vacuum overnight to ensure complete removal of the organic solvent. This film was then rehydrated with PBS pH 7.4 under hydrodynamic flow for a couple of hours (Bangham et al., 1967).

Table (1): Composition of RIV liposomes

Formula No	PL (mg)	RIV (mg)	CS %W/V	CH (mg)	TW 80 (mg)	SP 80 (mg)
RIV-CL1	60	10	0.10	40	0	0
RIV-CL2	60	10	0.20	40	0	0
RIV-CL3	60	10	0.40	40	0	0
RIV-CL4	60	10	0.50	40	0	0
RIV-TFL1	85	10	0.10	0	15	0
RIV-TFL2	85	10	0.20	0	15	0
RIV-TFL3	85	10	0.40	0	15	0
RIV-TFL4	85	10	0.50	0	15	0
RIV-SFL1	85	10	0.10	0	0	15
RIV-SFL2	85	10	0.20	0	0	15
RIV-SFL3	85	10	0.40	0	0	15
RIV-SFL4	85	10	0.50	0	0	15

It is well known that the stability of liposomes is critical to establish their safe and effective use and is dependent on both formulation and manufacturing method parameters (Caddeo et al., 2017). To minimize disruptive influences, the formation of a polymeric membrane (as CS in the current study) surrounding the liposome has been described by several authors (Bossy-Nobs et al., 2005; Kozhikhova et al., 2018; Ruysschaert et al., 2004).

In the current study, the chosen RIV formulations were subjected to a 6 months shelf storage stability testing. The formulations were packaged in tightly closed, amber color glass bottles and were stored in a refrigerator at 4 °C. At zero time and after one month, three months and six months, samples were withdrawn and PS, PDI, ZP and EE% were measured.

In- vitro RIV release studies from the stored RIV formulations were also conducted (under the same conditions and sampling times employed at zero time) to assess the effect of storage on RIV in vitro release. In vitro release results were subjected to analysis using different release kinetic models (zero order, first order and Higuchi diffusion models) and the kinetic parameters were determined and compared as previously discussed.

C. Drug Content

To assess the chemical stability of RIV in the formulations on storage, the drug content in the formulations was determined at 0, 1, 3 and 6 months of storage at 4° C. The degradation kinetic order of RIV in the selected formulations was determined employing graphical representation methods. Drug content data were plotted on different kinetic models (zero order, first order and second order) as described in table (2). The best fitting model was selected depending on the correlation coefficient values

Table (2): Kinetic treatments for the in-vitro drug release in the stability study

Order	X-axis	Y-axis	Slope	Intercept	$t_{1/2}$	Rate equation
Zero	(t)	(x)	(k)	0	(a/2k)	(x) = kt
First	(t)	Log (a-x)	-(k/2.3)	Log a	(0.693/k)	Log [(a)/(a-x)] = [(kt)/(2.3)]
Second	(t)	1/(a-x)	(k)	1/a	(1/a k)	(x)/[a(a-x)] = k t

Formulations were not tested at higher temperatures because, as concluded from literature, nanoparticles dispersions show high degree of instability at higher temperatures. This includes an increase in PS, a decrease in EE%, gelation and aggregate formation (Van Tran et al., 2019; Worthen et al., 2016; Xie et al., 2019). Total drug content of the formulations was determined by dissolving CS-coated liposomes in methanol and their RIV content was measured by an HPLC method (Guo et al., 2003).

D. HPLC Analysis

For determination of drug content in the formulations, CS coated liposomes were dissolved in methanol, cool centrifuged and the supernatant was evaporated under nitrogen, re-dissolved in the mobile phase. The resulting solution was analyzed employing a validated RP-HPLC method for RIV concentration (Çelebier et al., 2013). Separations were carried on a Thermo 5 µm C18 100 Å LC Column (250 x 4.6 mm). The flow rate was 1mL min-1 while using isocratic elution with ACN:Water (55:45 v/v) mixture. Injection volume was 100 µL and UV detection was performed at 249 nm. Peak identity was confirmed by retention time comparison.

IV. RESULTS AND DISCUSSION

The data in table (3) illustrates the results of the physicochemical characterization of the prepared formulations. On the basis of these results, best formulations were chosen from each category based on a rank order to investigate their stability.

Table (3): Results of the physicochemical characterization of the formulations at zero time

Formula No	PS (nm)	PDI	ZP (mV)	EE%
RIV-CL1	118.5 ± 2.26	0.73 ± 0.149	1.83± 0.199	98.13± 0.011
RIV-CL2	128.27 ± 6.67	1 ± 0	2.17± 0.213	97.08± 0.004
RIV-CL3	92.1 ± 5.92	0.712 ± 0.095	3.973± 0.565	97.6± 0.013
RIV-CL4	120.33 ± 9.92	1 ± 0	6.357± 0.477	96.62± 0.003
RIV-TFL1	130.83 ± 15.95	0.421 ± 0.078	4.513± 0.347	91.95± 0.009
RIV-TFL2	105.67 ± 1.55	0.331 ± 0.009	5.67± 1.202	96.07± 0.014
RIV-TFL3	145.83 ± 8.98	0.369 ± 0.098	7.707± 0.698	96± 0.003
RIV-TFL4	114.67 ± 8.07	0.394 ± 0.086	8.973± 1.261	94.47± 0.004
RIV-SFL1	145.8 ± 6.12	0.59 ± 0.026	1.933± 0.355	98.93± 0.009
RIV-SFL2	125.53 ± 3.39	0.537 ± 0.109	2.88± 0.329	98.03± 0.001
RIV-SFL3	133.63 ± 12.96	0.697 ± 0.216	3.73± 0.831	90.52± 0.019
RIV-SFL4	524.77 ± 20.58	0.536 ± 0.021	6.21± 0.775	91.67± 0.008

It was reported that classic liposomes are poorly stable (degradation, aggregation and fusion) and have a tendency to leakage of the entrapped drug during storage (Hao et al., 2017). CS coated liposomal surfaces showed proven efficacy to enhance the stability of liposomes, and overcome the above-mentioned drawbacks (Karewicz et al., 2013; Liu et al., 2013; Liu et al., 2016).

A. Results of PS Assessment

At zero time, the determined PS for RIV-CL3 was 92.1 nm, 80.02 nm after 1 month, 92.477 nm after 3 months and 105.063 after 6 months. The determined PS for RIV-SFL2 exhibited a similar behavior, that it was 125.533 nm on day 1, 121.198 nm after 1 month, 140.973 nm after 3 months and 143.396 nm after 6 months. As for formula RIV-TFL2, PS was found to be 105.667 nm on day one, 94.639 nm after 3 months and 111.373 nm after 6 months. Each formula was measured in a triplicate and the results were expressed as mean values. There was no visible sedimentation or particles in any sample during this period.

Table (4): Effect of storage period on the PS and PDI

Time (month)	Particle Size (nm)			Polydispersity Index		
	RIV-CL3	RIV-TFL2	RIV-SFL2	RIV-CL3	RIV-TFL2	RIV-SFL2
0	92.100	105.667	125.533	0.712	0.331	0.538
1	80.021	94.639	121.198	0.705	0.330	0.534
3	92.477	111.373	140.973	0.698	0.295	0.528
6	105.063	118.917	143.396	0.687	0.299	0.523

According to literature, classical uncoated liposome usually exhibits an increase in liposome particle size during storage. This behavior was not observed in CS coated liposomal formulations. This can be attributed to the ability of CS to stabilize the liposomes against fusion. This can explain the stability of the particle sizes of the coated formulations on storage. These results come in close agreement with the previous findings which reported a similar behavior of CS coated liposomes on storage (Henriksen et al., 1997; Liu and Park, 2010).

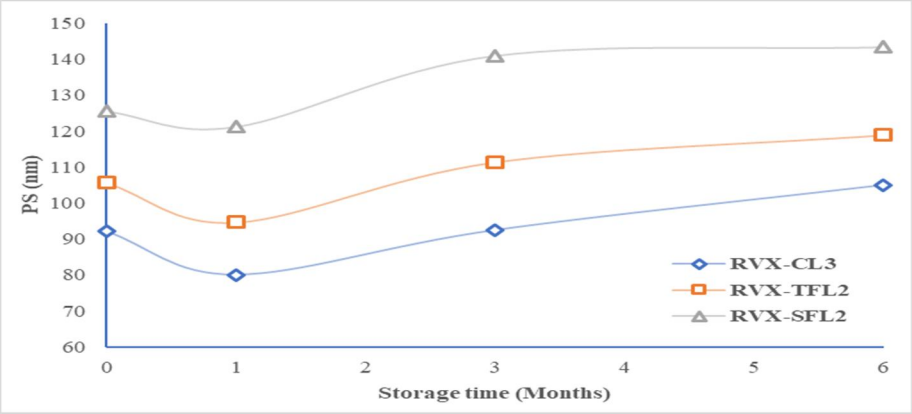

Fig. 1 Effect of storage on the PS of formulations RIV-CL3, RIV-TFL2 and RIV-SFL2

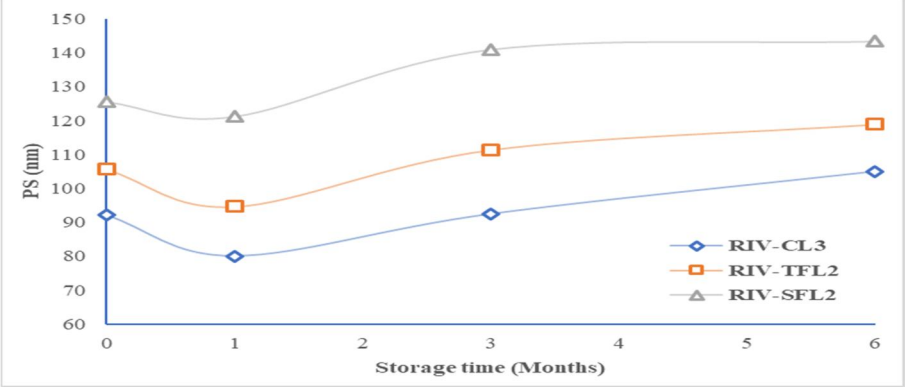

Fig. 1 Effect of storage on the PS of formulations RIV-CL3, RIV-TFL2 and RIV-SFL2

B. Results of PDI assessment

As for formula RIV-CL3, the PDI value dropped from 1 at day 1 to 0.756 after 6 months. The determined PS for RIV-TFL2 was 105.667 nm at zero-time, 96.639 nm after 1 month, 111.373 nm after 3 months and 118.917 nm after 6 months. The PDI for RIV-TFL2 dropped from 0.331 at zero time to 0.299 after 6 months. The PDI for RIV-SFL2 was 0.538 at zero time and dropped to 0.523 after 6 months

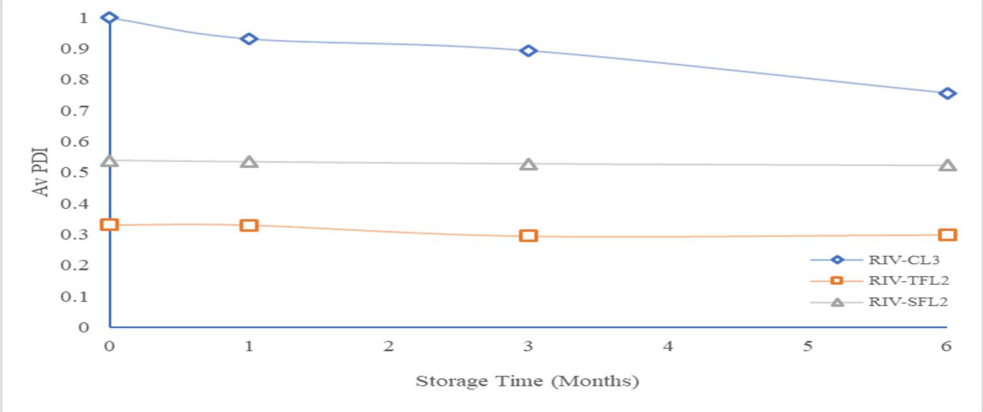

Fig. 3 Effect of storage on the PDI of formulations RIV-CL3, RIV-TFL2 and RIV-SFL2

C. Effect of storage on zeta potential and Entrapment Efficiency of RIV-CL3, RIV-TFL2 and RIV-SFL2

The data in table (5) and the charts in figures (4-5), demonstrate the effect of storage on the ZP and the EE% of the selected formulations. As the data shows, the selected formulations were generally stable on storage and no prominent change was observed on the mentioned two parameters.

Table (5): Effect of storage period on the ZP and EE% of RIV-CL3, RIV-TFL2 and RIV-SFL2

Time (month)	Zeta Potential (mV)			Entrapment Efficiency %		
	RIV-CL3	RIV-TFL2	RIV-SFL2	RIV-CL3	RIV-TFL2	RIV-SFL2
0	3.973	5.670	2.880	97.60	96.07	98.03
1	3.901	5.612	2.800	97.51	96.00	98.00
3	3.887	5.548	2.750	97.48	95.94	97.84
6	3.845	5.521	2.732	97.41	95.87	97.63

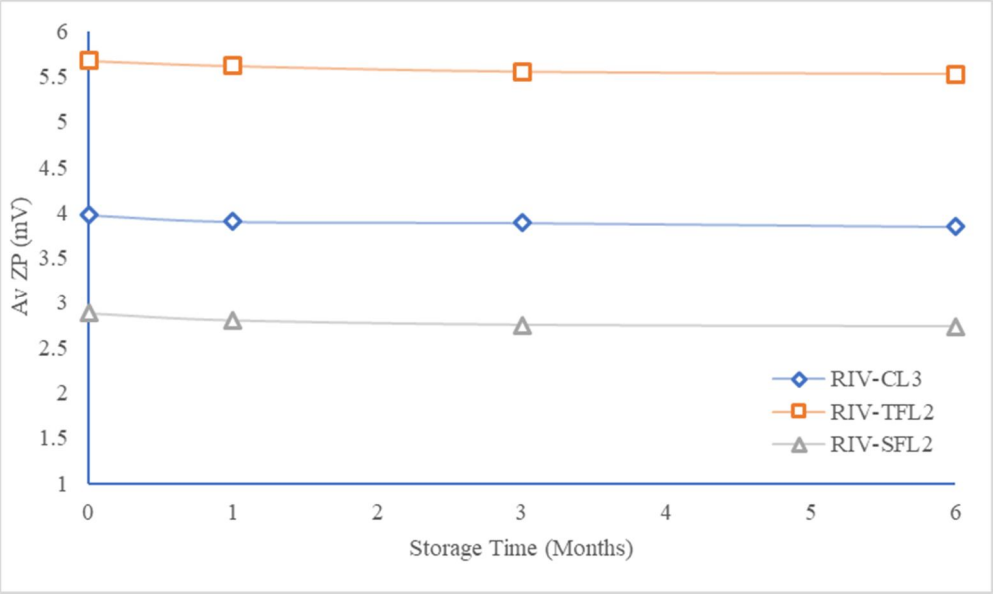

Fig. 4 Effect of storage on ZP of formulations RIV-CL3, RIV-TFL2 and RIV-SFL2

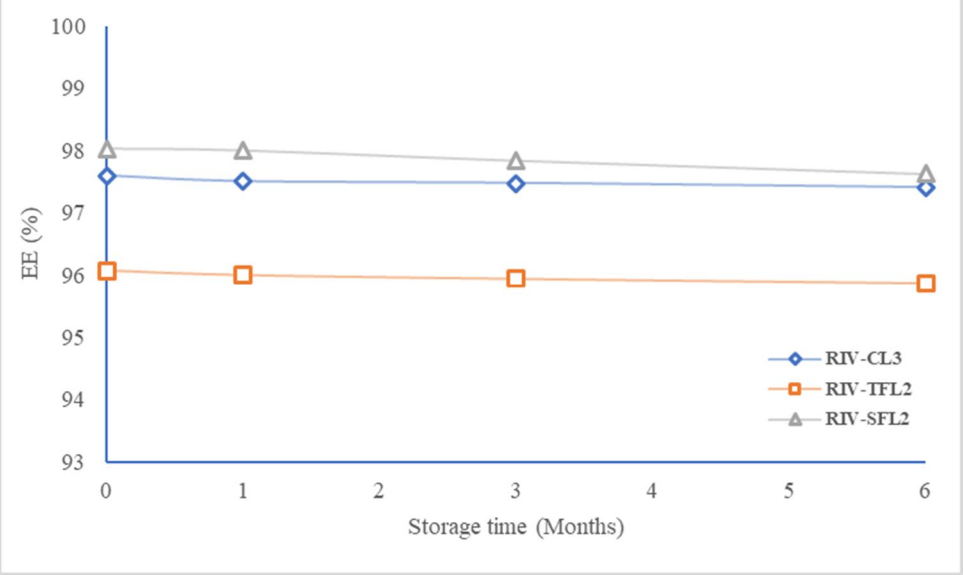

Fig. 5 Effect of storage on the EE% of formulations RIV-CL3, RIV-TFL2 and RIV-SFL2

D. Effect of storage on the in vitro release of RIV formulations

To ensure the data gained from studying the physical stability of the selected formulations, the *in vitro* release experiments were performed using the same formulations after 6 months of storage at 4 °C. Data in table (6) and figures (6-8) shows a comparison between the release profiles of each of the selected formulations on the same day of preparation and after 6 months of storage.

The data confirmed the results obtained from studying the physical stabilities of the formulations and no prominent differences between the *in-vitro* release profiles of the fresh and stored formulations were observed. The slight increase in the *in-vitro* release can be attribute to the minor diffusion of the entrapped drug that is expected to occur upon storage.

TABLE (6): Effect of storage period on the *in-vitro* release of RIV formulations

Time (hr)	RIV-CL3		RIV-TFL2		RIV-SFL2	
	CR% at Zero time	CR%After 6 months	CR% at Zero time	CR%After 6 months	CR% at Zero time	CR%After 6 months
0	0.00	0.00	0.00	0.00	0.00	0.00
0.5	12.48	13.89	25.18	28.34	17.91	19.35
1	18.43	19.87	31.81	36.32	27.01	30.56
2	19.25	21.58	41.19	46.48	28.54	32.12
4	24.95	26.45	55.93	56.78	28.68	33.56
5	31.76	33.12	57.84	59.27	35.64	37.78
6	32.61	34.67	64.53	66.29	37.86	38.56
7	34.22	36.36	69.13	70.37	37.99	39.34
8	37.23	39.34	70.43	73.45	40.69	43.89
12	37.47	40.16	70.55	76.24	45.01	48.27
24	43.06	45.58	84.00	86.45	66.14	70.85

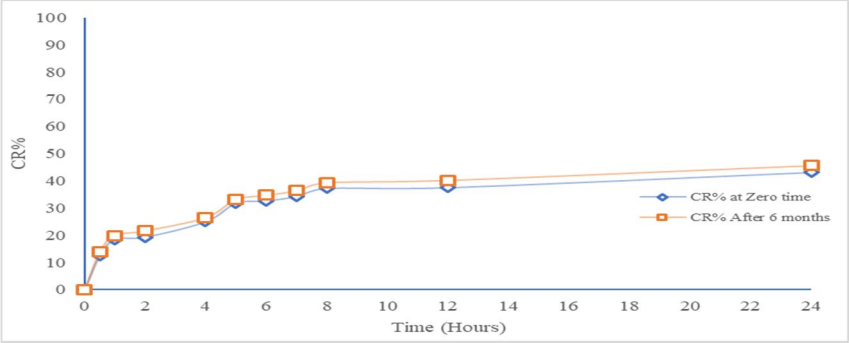

Fig. 6 In-vitro release of RIV-CL3 formula at zero time and after six months of storage

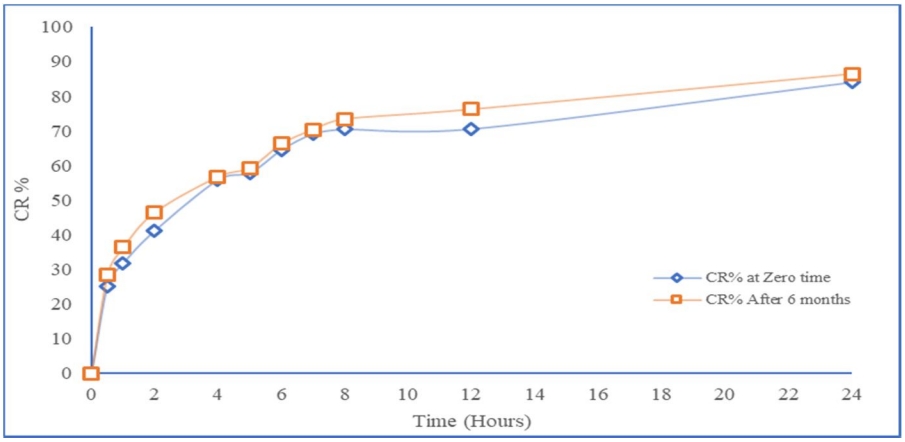

Fig. 7 In-vitro release of RIV-TFL2 formula at zero time and after six months of storage

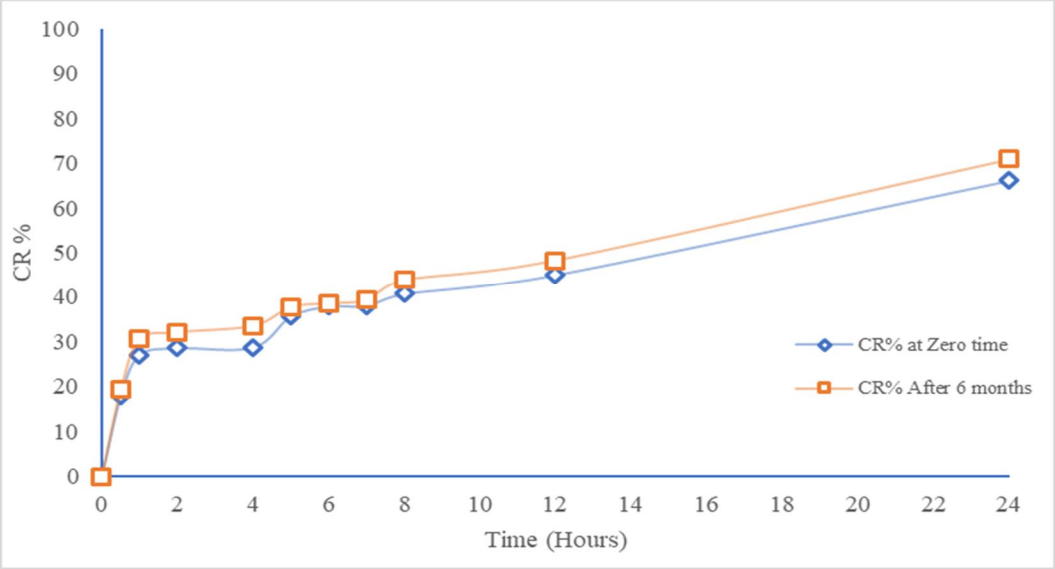

Fig. 8 In-vitro release of RIV-SFL2 formula at zero time and after six months of storage

The correlation coefficients for the selected formulations with the tested parameters on storage are listed in table (7). As the data shows some parameters have direct proportionality and the others have inverse proportionality.

Generally, PS has direct proportionality with the IVR at time zero and after 6 months of storage. However, PS has inverse proportionality with PDI, ZP & EE%. In contrast, PDI has direct proportionality with ZP & EE% and inverse proportionality with IVR at zero time and after 6 months of storage. ZP has direct proportionality with EE% and inverse proportionality with IVR at zero time and after 6 months of storage. EE% showed inverse proportionality with IVR at both zero time and after 6 months of storage. Finally, IVR at zero time showed direct proportionality with the IVR after 6 months of storage.

Table (7): Correlation coefficients for the physico-chemical properties and *in vitro* release after six months of storage.

RIV-CL3					
	PS	PDI	ZP	EE	IVR_0
PDI	-0.70415				
ZP	-0.44779	0.950118			
EE	-0.5306	0.976791	0.994845		
IVR_0	0.331107	-0.90221	-0.99138	-0.97357	
IVR_6	0.333806	-0.90425	-0.99232	-0.97468	0.999498
RIV-TFL2					
	PS	PDI	ZP	EE	IVR_0
PDI	-0.80823				
ZP	-0.67238	0.910515			
EE	-0.69829	0.856007	0.984527		
IVR_0	0.51752	-0.67777	-0.91543	-0.95871	
IVR_6	0.50271	-0.74158	-0.95257	-0.9692	0.988163
RIV-SFL2					
	PS	PDI	ZP	EE	IVR_0
PDI	-0.89075				
ZP	-0.77923	0.948629			
EE	-0.89767	0.970494	0.845413		
IVR_0	0.758309	-0.95756	-0.88243	-0.95989	
IVR_6	0.761226	-0.94968	-0.85703	-0.96589	0.998353

E. Kinetic stability study of RIV formulations upon storage

To assess the chemical stability of the drug in the selected formulations on storage, the drug content in the formulations was determined on 0 time and after 1, 3 and 6 months of storage at 4 °C by the HPLC method described in the methodology section. The obtained data was illustrated on table (8) and expressed as percentages from the value of the drug content determined in the freshly prepared formulations.

Table (8): The percent of RIV un-degraded after shelf storage for six months

Time (month)	Mean of RIV % un-degraded		
	RIV-CL3	RIV-TFL2	RIV-SFL2
	4 ˚C (refrigerator)		
0	99.704	99.035	99.825
1	99.643	97.346	98.438
3	98.686	96.613	97.258
6	96.128	95.856	96.012

The degradation kinetic order of RIV in the selected formulations was determined employing graphical representation methods. Drug content data were plotted on different kinetic models (zero order, first order and second order) and the best fitting model were selected depending on the correlation coefficient values. The degradation kinetics data indicated that degradation of RIV on storage followed zero order. The related data is represented in tables (9) and (10).

Table (9): Kinetic parameters for the stability study in the selected RIV formulations

Formula No.	Kinetic Parameters					
	At 4 °C (refrigerator)					
	Order or mechanism	Intercept	Slope	correlation coefficient (r)	K	$t_{1/2}$
RIV-CL3	Zero	-0.07995	0.615881	0.973181	0.615881	81.18452
	First	2.000393	-0.00273	-0.97244	-0.00629	-110.187
	Second	0.00999	6.42E-05	0.971685	6.42E-05	155.7401
RIV-TFL2	Zero	1.620357	0.466857	0.919044	0.466857	107.0991
	First	1.9929	-0.00208	-0.91104	-0.0048	-144.393
	Second	0.010165	4.93E-05	0.913024	4.93E-05	202.7349
RVS-SFL2	Zero	0.622881	0.597548	0.989918	0.597548	83.67534
	First	1.997299	-0.00265	-0.97134	-0.00611	-113.367
	Second	0.010062	6.25E-05	0.972737	6.25E-05	159.9516

TABLE (9): The calculated correlation coefficients for stability study of RIV formulations

RIV Formula	Correlation coefficients (r)		
	Zero-order	First-order	Second-order
	At 4 °C (refrigerator)		
RIV-CL3	0.973181	-0.97244	0.971685
RIV-TFL2	0.919044	-0.91104	0.913024
RIV-SFL2	0.989918	-0.97134	0.972737

According to the obtained results, the shelf lives (t_{90}) of the selected formulations were calculated. The results showed that formula RIV-TFL2 was the most stable one having a shelf life of about 21.4 months followed by formulations RIV-SFL2 and RIV-CL3 whose shelf lived were found to be 16.7 and 16.2 months respectively. The obtained results are illustrated in table (11).

TABLE (11): The determined t_{90} for RIV formulae after stability study

RIV Formula	t_{90}	
At 4 °C (refrigerator)		
	Months	Years
RIV-CL3	16.2369	1.35
RIV-TFL2	21.41982	1.78
RIV-SFL2	16.73507	1.39

V. CONCLUSIONS

The effect of storage on both physical and chemical stabilities of the selected RIV loaded liposomes coated with CS was investigated. The stability of the formulations was demonstrated, and no sedimentation was observed throughout the storage period. According to the obtained results, formula RIV-TFL2 (containing 15% TW80 and coated with 0.2% CS solution) was the most stable one having a shelf life of about 21.4 months followed by formulations RIV-SFL2 and RIV-CL3 whose shelf lived were found to be 16.7 and 16.2 months respectively. Overall, CS coated liposomes can be considered an effective and reliable approach for the design of stable oral RIV dosage forms.

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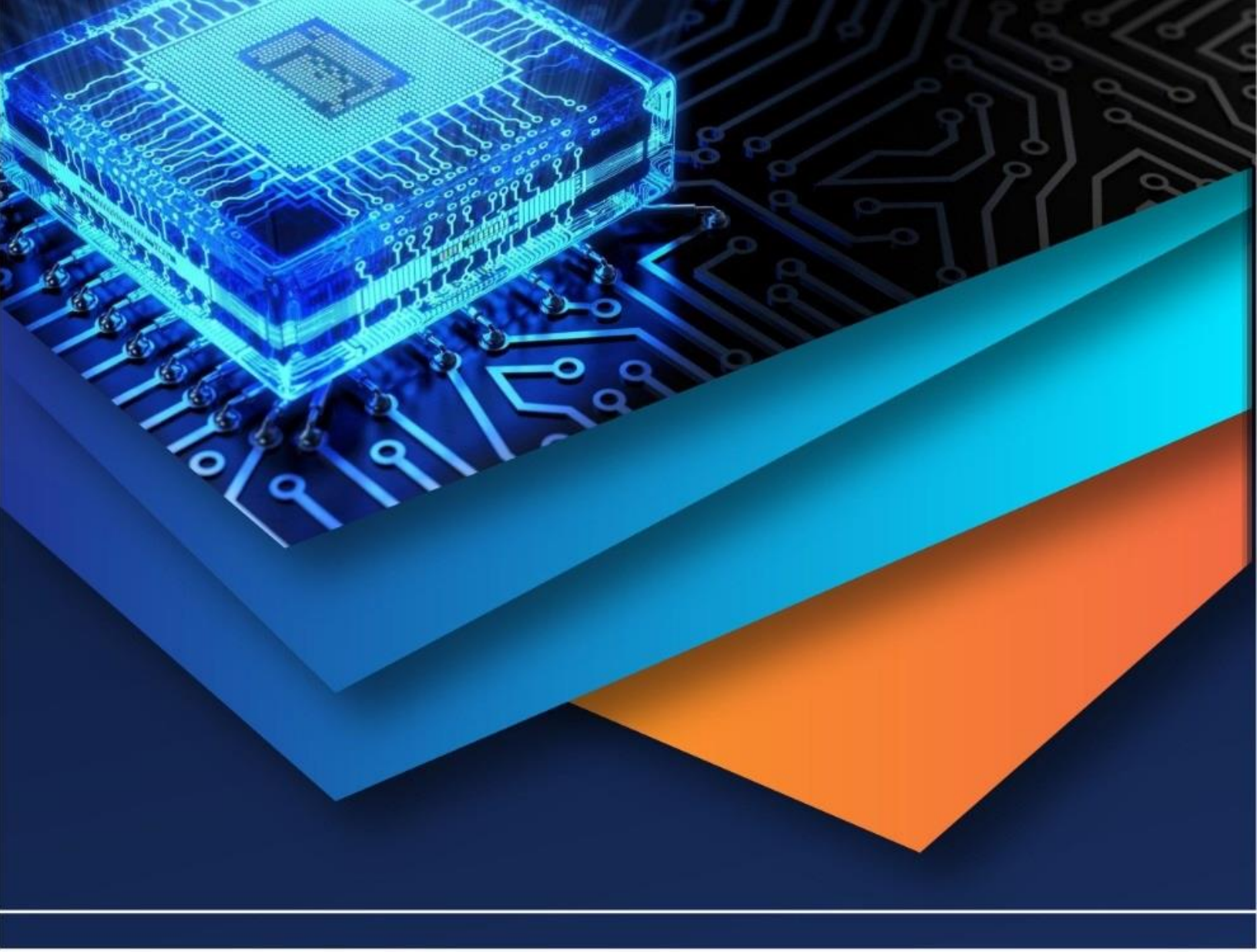

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