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FOLATE ANCHORED SAQUINAVIR ENTRAPPED PLGA NANOPARTICLES: STABILITY STUDY

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ABSTRACT

Polymers are the most commonly explored materials for constructing nanoparticle-based drug carriers. Polymeric nanoparticles can be easily manipulated without the loss of their physical, chemical and biological properties. Freeze-drying of nanoparticles has been considered as a good technique to improve the long-term stability. In this study, we have described stability study of formulations relative to pure drug and stability analysis at different storage conditions.Our main aim in the present investigation was to confirm drug Saquinavir (SQV), polymer Polylactide co-glycolide (PLGA), ligand Folic acid (FA) characteristics and determine stability of Saquinavir (SQV), polymer (PLGA) and synthesized PEGylated PLGA NPs & SQV-loaded FA anchored PEGylated PLGA NPs for tumor targeting.Results: The drug is stable at normal storage medium condition and targeted nanoparticles were found to be most stable at the refrigerated conditions of storage. The developed formulation can be successfully used as potential delivery vehicle for delivery of SQV.

INTRODUCTION

Developing an efficient drug delivery system has been the area of interest amongst the researchers in the past two decades. Polymers are the most commonly explored materials for constructing nanoparticle-based drug carriers with controlled drug release and increased aqueous solubility [1,2] because they have higher affinity for preferential accumulation in certain solid tumors through leaky endothelial tissue surrounding the tumor through EPR effect [3,4]. Polymeric nanoparticles can be easily manipulated without the loss of their physical, chemical and biological properties. The most promising approach is the surface modification of the carrier by dysopsonic polymer such as poly (ethylene glycol) (PEG) [5,6].

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The nanoparticulate delivery systems not only tend to increase the stability of drugs but also are able to control the release of drug molecules over a longer period [7].

In this study, stability study for SQV,PLGA polymer, synthesized folate legand via a polyethylene glycol spacer (PEG) and SQV loaded non-targeted PLGA NP were performed for the targeted delivery of SQV-FOL (Folate) decorated nanoparticles for targeting to tumors [8]. Folic acid (FA) has chosen as targeting moiety because it participates in the biosynthesis of nucleotide bases and are available in pteroyl-L-glutamic acid, pteroyl-L-glutamate and pteroyl mono glutamic acid forms. Folate receptors (FRs) have been frequently over-expressed in a wide range of tumors and generally present in caveolae membrane protein [9-11]. Folic acid retains it's receptor-binding and endocytotic properties when covalently linked to a wide variety of molecules [12].

The principle can be used to greatly improve the function of the nanoparticles in cancer therapy through the attachment of tumor specific targeting moieties, directed at cell surface markers unique to the cancer cell.

In the previous study we have developed and characterized SQV-loaded FA anchored PEGylated PLGA NPs for tumor targeting [2].

The purpose of the present work was to evaluate the comparative stability of the SQV, PLGA polymer, SQV loaded PLGA and SQV loaded FA anchored PLGA nanoparticles, after 3 months at different conditions.

MATERIALS & METHODS

In this study following materials were used.

Saquinavir was obtained as a gift sample from Hoffman La Roche (Mannheim, Germany). Di methyl sulfoxide (Rankem), Folic acid (Himedia), Poly (D, L-Lactide –Co-Glycolide) (lactide:glycolide 50:50, mol wt 40,000-75,000) (Sigma), Poly ethylene glycol bis amine (Sigma), Phasphate buffer saline tablets pH 4 (Himedia), Phasphate buffer saline tablets pH 7 (Himedia), sodium azide (Himedia) All reagents and solvents were analytical grade and were used as received.

Stability studies of drug

SQV (10mg) was tested for stability preserved in 10ml of buffer solution (PBS, pH 7.4) with 0.1 % *(w/v)* sodium azide for a period of three months on storing it at three different temperature refrigerating temperature (4±2^{0}C), room temperature (25 ± 2^{0}C) and elevated temperature (37±2^{0}C). The Drug was stored in amber coloured screw capped vials in dark and colourless vials in light via separately at 4±2^{0}C, 25±2^{0}C and 37 ± 2^{0}C for one month, two month and three months.

Vials were placed in refrigerator for 4±2^{0}C, humidity control chambers (REMI, Mumbai, India) for 25±2^{0}C and incubator (YSI–438 Yorco Incubator Bacteriological, New Delhi, India) 37±2^{0}C. The samples were analysed initially and periodically after one, two and three months under following parameters.

1. Media pH

The pH value of media is measured by using pH meter (Microprocessor pH meter 1012, ESICO, USA). The change of pH if any, was recorded.

2. **Turbidity**

Turbidity was evaluated by turbidometer (Systronics, India). The change in turbidity, if any was recorded.

3. Change in color and consistency by visual observations

It was characterized by visual observations. The change of color & consistency, if any was recorded.

Stability studies of formulations

Briefly, lyophilized SQV-PLGA and SQV-FOL-PEG-PLGA NP's (10mg) were redispersed separately in 10ml of buffer solution (PBS, 7.4) in both amber coloured and colourless screw capped vials, then labelled and preserved in PBS at pH 7.4 with 0.1 % *(w/v)* sodium azide for period of one, two and three months. Amber coloured and colourless vials contains nanoparticles formulation were incubated at refrigerating conditions 4±2°C, room temperature (RT) 25±2^{0}C and elevated temperature 37±2° for a period of one, two and three months. The stability was performed as mentioned in earlier section.

RESULT & DISCUSSION

Drug stability tested at three different temperatures in aqueous solution for three months. The drug showed relatively better stability at temperature 4 ± 2^{o}C, 25± 2^{o}C comparatively than at 37 ± 2^{o}C. Stability study results have shown the best stability at 4 ± 2^{o}C in dark (Table 1). But after 3 months storage at 4 ± 2^{o}C in light, slight precipitation of drug was observed than dark in aqueous solution. In light more precipitation was observed at 25± 2^{0}C & 37 ± 2^{0}C shown in Table 2 and 3. The amount of precipitate was more at 37 ± 2^{0}C than at 25± 2^{0}C. Drug stability study has shown that there was no remarkable change in the drug at 4±2^{0}C, 25±2^{0}C. This indicated that drug is stable at normal storage medium condition.

Table 1. Stability study of drug at 4±2^{0}C temperature for three months

Parameters	SQV One month Dark	SQV One month Light	SQV Two months Dark	SQV Two months Light	SQV Three months Dark	SQV Three months Light
pH	7.4	7.2	7.4	7.4	7.4	7.2
Turbidity	-	-	-	-	-	-
Change in color	-	-	-	-	-	-
Consistency	-	-	-	-	-	-

- = indicate no change + = indicate smaller change +++ = indicate enough change

Table 2. Stability study of drug at 25 ± 2^{0}C temperature for three months

Parameters	SQV One month Dark Light		SQV Two month Dark Light		SQV Three month Dark Light	
pH	7.4	7.2	7.4	7.2	7.4	7.2
Turbidity	-	-	-	-	+	+++
Change in color	-	-	-	-	-	-
Consistency	-	-	-	-	-	-

- = indicate no change + = indicate smaller change +++ = indicate enough change

Table 3. Stability study of Drug at 37 ± 2^{0}C temperature for three months

Parameters	SQV One month Dark Light		SQV Two month Dark Light		SQV Three month Dark Light	
pH	7.4	7.2	7.4	7.4	7.2	7.2
Turbidity	-	+	-	+	+	+++
Change in color	-	-	-	+	-	+
Consistency	-	-	-	-	-	-

- = indicate no change + = indicate smaller change +++ = indicate enough change

The prepared nanoparticles formulation tested for stability on storing them at three different temperature 4 ± 2^{0}C and 25 ± 2^{0}C, 37 ± 2^{0}C. The parameters observed are recorded in Table 4 - 12. pH of all NPs formulations stored at 4±2^{o}C and showed no significant changes in pH, but at room temperature (25±2^{0}C) and elevated temperature (37±2^{0}C) pH was found to be decrease to some extent. It may be attributed to a change in ionic strength because of degradation of the copolymer at this temperature.

Table 4. Nanoparticles formulations stability study stored at 4 ± 2^{0}C for one month.

Parameters	SQV-PLGA NPs One month		SQV-FOL-PEG-PLGA NPs One month	
	Dark	light	Dark	light
pH	7.4	7.2	7.4	7.4
Turbidity	-	-	-	-
Change in color	-	-	-	-
Consistency	-	-	-	-

- = indicate no change.

Table 5. Nanoparticle formulations stability study stored at 25 ± 2°C for one month.

Parameters	SQV-PLGA NPs One month		SQV-FOL-PEG-PLGA NPs One month	
	Dark	light	Dark	Light
pH	7.4	7.4	7.4	7.1
Turbidity	-	-	-	-
Change in color	-	-	-	-
Consistency	-	-	-	-

- = indicate no change

Table 6. Nanoparticles formulations stability study stored at 37±2°C for one month.

Parameters	SQV-PLGA NPs One month		SQV-FOL-PEG-PLGA NPs One month	
	Dark	Light	Dark	Light
pH	7.0	6.5	6.8	6.6
Size	-	-	-	-
Turbidity	-	-	-	-
Change in color	-	-	-	-
Consistency	-	-	-	-

- = indicate no change

Table 7. Nanoparticles formulations stability study stored at 4 ± 2°C for two months.

Parameters	SQV-PLGA NPs Two months		SQV-FOL-PEG-PLGA NPs Two months	
	Dark	light	Dark	light
pH	7.0	6.8	7.2	7.0
Turbidity	-	-	-	-
Change in color	-	-	-	-
Consistency	-	-	-	-

- = indicate no change

Table 8. Nanoparticles formulations stability study stored at 25 ± 2°C for two months.

Parameters	SQV-PLGA NPs Two month		SQV-FOL-PEG-PLGA NPs Two month	
	Dark	light	Dark	light
pH	7.2	6.8	7.2	7.0
Turbidity	-	-	-	-
Change in color	-	-	-	-
Consistency	-	-	-	-

- = indicate no change

Table 9. Nanoparticles formulations stability study stored at 37 ± 2°C for two months.

Parameters	SQV-PLGA NPs Two month		SQV-FOL-PEG-PLGA NPs Two month	
	Dark	light	Dark	light
pH	6.8	6.5	6.5	6.4
Turbidity	-	-	-	-
Change in color	-	-	-	-
Consistency	-	+	-	-

- = indicate no change + = indicate smaller change

Table 10. Nanoparticles formulations stability study stored at 4±2°C for three months.

Parameters	SQV-PLGA NPs Three months		SQV-FOL-PEG-PLGA NPs Three months	
	Dark	light	Dark	light
pH	6.8	6.5	7.0	6.8
Turbidity	-	-	-	-
Change in color	-	-	-	-
Consistency	-	-	-	-

- = indicate no change.

Table 11. Nanoparticles formulations stability study stored at 25 ± 2°C for three months.

Parameters	SQV-PLGA NPs Three month		SQV-FOL-PEG-PLGA NPs Three month	
	Dark	light	Dark	Light
pH	6.2	6.0	6 .4	6.2
Turbidity	-	+	-	+
Change in color	+	++	+	++
Consistency	+ +	++	+	++

- = indicate no change + = indicate smaller change ++ = indicate considerable change.

Table 12. Nanoparticles formulations stability study stored at 37 ± 2°C for three months.

Parameters	SQV-PLGA NPs Three month		SQV-FOL-PEG-PLGA NPs Three month	
	Dark	Light	Dark	light
pH	6.0	5.8	6.2	6.0
Turbidity	-	++	-	+
Change in color	+	++	-	+
Consistency	+	++	+	++

- = indicate no change + = indicate smaller change ++ = indicate considerable change.

No significant change in color and consistency were observed in case of both drug loaded PLGA NPs at 4± 2^0C, 25± 2^0C, 37± 2^0C after one months of storage. Slight change in color and consistency was observed at 4± 2^oC after three months storage. Turbidity and consistency increased with increasing temperature. Nanoparticle formulations stored at 37± 2^oC showed considerable change in color and consistency due to high temperature.

Ligand anchored drug loaded (SQV-FOL-PEG-PLGA) NPs stored at 37± 2^0C, for three months showed high turbidity than drug loaded (SQV-PLGA) NPs, when kept both in dark and light. The storage at 37± 2^oC might have induced polymerization tendency of free groups by degradation of structure which resulted in the appearance of precipitation and turbidity. The NPs formulation (SQV-PLGA NPs & SQV-FOL-PEG-PLGA NPs) showed better stability with regards to pH, turbidity, change in color and consistency when stored at dark as well as refrigerated conditions. Thus storage of NPs formulation in amber colored vials as well as refrigerated conditions is recommended.

In this study SQV loaded FOL targeted nanocarriers have been developed to circumvent some of these problems, to reduce side effects of SQV. SQV nontargeted PLGA and folate targeted PLGA NPs were synthesized and optimum conditions for storage of nanoformulations have been found.

CONCLUSION

The focus in the present study has been on the stability of nanocarriers and molecules that can selectively target tumors, to explore anticancer activity in selected antiviral drug and to highlight the challenges in translating some of the basic research to the clinic.

Stability studies of drug and drug loaded nanopartilcles for three months revealed that NPs formulation stored at 4 ± 2^0C in dark were more stable than those stored at 25± 2^0C or 37 ± 2^0C in light.

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