Profiling In-Vitro Release of Verteporfin from VISUDYNE Liposomal Formulation and Investigating the Kinetics of Human Serum Albumin (HSA) - Verteporfin Complex Formation

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Purpose

Develop an *in vitro* drug release method for VISUYDNE (verteporfin) liposomal formulation to better mimic *in vivo* conditions and evaluate the interactions of verteporfin with human serum albumin (HSA).

Introduction

VISUYDNE is an intravenously administered liposomal formulation of verteporfin which is used for photodynamic therapy associated with age-related macular degeneration (AMD).

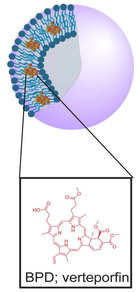

Figure 01. Schematic illustration of VISUDYNE liposome
BPD: Benzoporphyrin derivative
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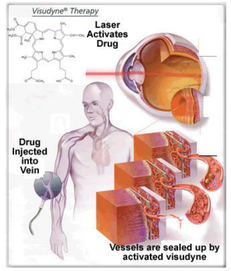


Figure 02. Schematic illustration for treatment for AMD using verteporfin

- Egg phosphatidylglycerol (EPG) and dimyristoyl phosphatidyl choline (DMPC) are two critical lipid excipients in VISUDYNE based on the drug product labelling.
- EPG is a natural, unsaturated phospholipid which has a phase transition temperature (Tm) around 10°C while DMPC is a synthetic, saturated lipid with Tm at 23°C.
- Hydrophobic verteporfin partitions into lipid bilayer.
- Both in vivo and in vitro studies have revealed immediate and complete transfer of verteporfin to plasma protein.
- An in vitro release method of verteporfin liposome in 5% v/v fetal bovine serum (FBS) was reported in literature. However, this method measures the fluorescence quenching of the protein-bound verteporfin at a set time and does not provide kinetics of released verteporfin over time.
- Human serum albumin (HSA) is the most abundant protein found in the human blood with the concentration range of 35-50 g L^{-1}.

Therefore, we need to

- Develop a new in vitro drug release method to better mimic in vivo conditions and describe the release kinetics

- Obtain better understanding about interactions of verteporfin with HSA in vivo

Materials and Methods

Profiling drug release

- To mimic in vivo conditions after drug administration, HSA was added in the dissolution medium to simulate human blood. In vitro drug release profiles for VISUYDNE were generated by changing the molar ratios of verteporfin:HSA including 0.25, 0.5, 1, 2,5, 15 with respect to moles of Verteporfin.
- A capillary electrophoresis-based method was developed for the quantification of verteporfin released from VISUDYNE formulation.
- The complexation of verteporfin-HSA was detected at 428 nm.

Investigating kinetics of verteporfin-HSA complexation

- The intrinsic fluorescence of HSA was measured by exciting the protein solution at 290 nm and the emission spectra was recorded in the range of 300-500 nm.
- Liposomal formulation or verteporfin solution at a concentration range from 8.69 x10^{-9} to 3.47x10^{-7} mol dm^{-3} was used upon incubating with HSA (1.10 x 10^{-5} M) at the following temperatures: 22°C, 27°C, 32°C, 37°C and 42°C for 30 minutes.
- By using the Stern-Volmer equation, the number of binding sites and binding constant for verteporfin-HSA were calculated.
- For liposomal formulation total verteporfin concentration was used for the calculation and apparent binding constant was calculated.

$\mathbf{Log_{10}(F_0\text{-}F)/F = log_{10}\, k + n\ log_{10}\,[C]}$

Where F_0 and F = fluorescence without and with the quencher molecule, [C] = concentration of the quencher n = the number of binding sites, k = the binding constant.

$\mathbf{\ln kt = -\Delta H/RT + \Delta S/R}$

Where k= binding constant, ΔH= Enthalpy, ΔS= Entropy, R= Universal gas constant, T= Temperature

Change in Gibbs free energy can be calculated using following equation.

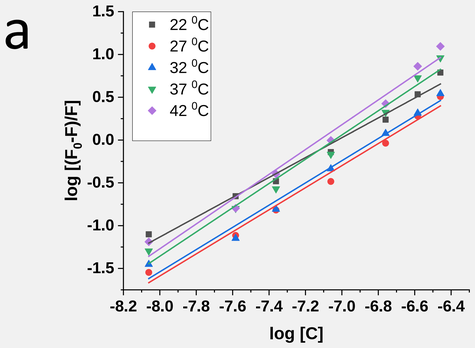
$\mathbf{\Delta G = \Delta H - T\Delta S}$

- Further, using the van 't Hoff equation, enthalpy, entropy, and Gibbs free energy were calculated for the complexation.

Capillary electrophoresis conditions

Capillary	Fused silica capillary column
Dimensions	75 µm inner diameter, and 72 cm length with high sensitivity cell
Sample injection	20 mbar, 30 s, hydrodynamic manner
Temperature	15 °C
λ_{max}	280 nm (for HSA) 428 nm (for Verteporfin)
Buffer	5 mM phosphate buffer+ 1% PEG-600
pH	7.4
Electric field	30 kV

Results and Discussion



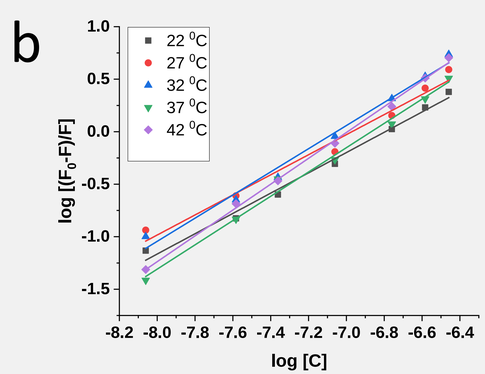


Figure 5. Verteporfin (API)-HSA Complexation Kinetics-Stern-Volmer Plot for liposomal formulation (a) and free verteporfin (b)

Table 2. Temperature dependent binding constants for liposomal verteporfin and fee verteporfin (API)-HSA Complexation

T (°C)	Binding sites		Binding constant	
	Liposomal	API	Liposomal*	API
22	0.97	1.1	3.672X10^6	1.396X10^8
22	0.97	1.1	3.672X10^6	5.321X10^8
27	0.96	1.2	4.581X10^6	6.998X10^8
32	1.10	1.2	5.984X10^7	9.376X10^9
37	1.16	1.4	8.790X10^7	2.104X10^{10}
42	1.23	1.4	4.017X10^8	1.396X10^8

*For liposomal formulation, total verteporfin concentration was used for the calculation and apparent binding constant was calculated.

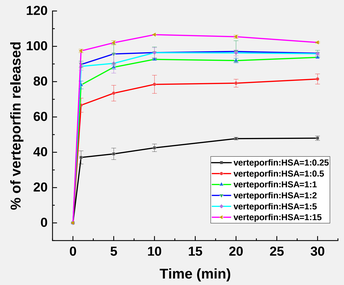


Figure 5. Verteporfin (API)-HSA ratio depended drug release profiles of VISUDYNE ((mean ± SD, N =3)

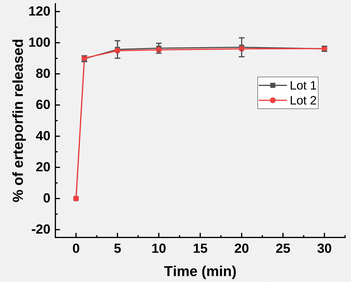


Figure 6. Verteporfin release profiles of two VISUDYNE Batches (pH=7.4, T= 37 °C, verteporfin:HSA=1:2)

Conclusions

- A capillary electrophoresis-based method was developed for the quantification of verteporfin released from VISUDYNE formulation.
- Considering the binding behavior between verteporfin and HSA and the goal to achieve complete drug release and minimal absorbance interference from HSA, we select verteporfin: HSA ratio 1:2 for further in vitro release testing of VISUDYNE.
- The binding molar ratio between verteporfin and HSA is around 1:1.
- The binding of verteporfin to HSA is a spontaneous exothermic process with a negative change in Gibbs free energy.
- The apparent binding constant of liposomal verteporfin into HSA is 100-fold less than that for free verteporfin, possibly due to partition of verteporfin in liposomal lipid domain.

Acknowledgements and Disclaimer

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