

## Erythrocyte uptake of drugs and its impact on volume of distribution ( $V_D$ ) determinations

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### Abstract

In most volume of distribution ( $V_D$ ) determinations the drug partitioned in to erythrocytes ( $C_{ery}$ ) occupying 45% of blood volume is disregarded. The  $V_D$  determinations can be erroneous on two accounts. The first is the indiscriminate reference to plasma ( $C_p$ ), whole blood ( $C_b$ ) or serum ( $C_s$ ) concentrations. The second is when  $C_{ery}$  values are not considered in calculations. Isolated erythrocytes were incubated in plasma water ( $C_{pw}$ ) represented by physiological saline drug solutions, the  $C_{pw}$ ,  $C_{ery}$  and  $C_b$  values were experimentally determined *in vitro*. Aberrations to the  $V_D$  determinations are demonstrated using both theoretically and practically determined values of  $C_{pw}$ ,  $C_{ery}$  and  $C_b$ . Widely varying  $V_D$  values 125 L to 2.55 L resulted when  $C_p$  data alone is used while the values differed marginally from 4.56 L to 5.53 L when  $C_b$  values were used for two setting using same amount of drug.

**Key words:** Volume of distribution, Erythrocyte drug concentration  $C_{ery}$ ,

### Introduction

The present study highlights the repercussions of indiscriminate use of drug blood concentration ( $C_b$ ), plasma concentration ( $C_p$ ) and serum concentration ( $C_s$ ). A plasma determination is sometimes referred to as blood concentration. The erythrocyte partitioned drug has so far evaded receiving due recognition<sup>1</sup>. This identifies a fourth concentration parameter, which is the erythrocyte concentration of drugs ( $C_{ery}$ ). This parameter is occasionally mentioned in the literature<sup>2</sup>. The  $C_{ery}$  values are sometimes over five times higher than the  $C_p$  values<sup>3</sup>.

Isolated erythrocytes were incubated *in vitro* setting with doxycycline<sup>4</sup>, chloramphenicol, rifampicin, oxytetracycline and chloroquine solutions of known strength. The  $C_{ery}$  and  $C_{pw}$  values were determined using standard curves ('Determination of Uptake of Selected Drugs by Red Blood Corpuscles, B. Sc. Pharmacy, Department of Chemistry,

University of Colombo, 2009 and Influence of Drugs Partitioned in to Red Blood Corpuscles on Volume of Distribution Determinations, University of Sri Jaywardenepura, B. Pharm program 2015).

The results were treated to demonstrate variations in volume of distribution values with and without taking into account  $C_{rbc}$  values. Similar variations were also demonstrated for situations where  $C_b$ ,  $C_p$  or  $C_s$  is used indiscriminately. The volume of distribution studies are understood with the aid of compartment models<sup>5</sup>.

## Materials and methods

### Model theoretical $V_D$ calculations based on plasma ( $C_p$ ), serum ( $C_s$ ) or whole blood ( $C_b$ ) concentrations:

The symbol  $C_{pw}$  is for *in vitro* studies without using blood. The total blood volume of an adult is considered to be 4.5 L. Since 55 % by volume of blood constitute plasma, the volume of plasma approximates 2.5 L.

A 25 mg of a drug X was administered by IV bolus to a subject resulting in an immediate plasma concentration of  $(25 \times 1000)/(2.5 \times 1000) = 10 \mu\text{g/ml}$ , prior to drug partitioning. The figures 1a - e for 100 ml of blood depict the five situations required for the demonstration of model calculations. Lowering effects on drug concentrations  $C_p$  and  $C_{ery}$  due to 40% drug partitioning in to tissues are shown in Figure 1e.

The volumes of packed erythrocytes: plasma is 45: 55 and calculations must account for this numerical unevenness.

Figure 1a shows a theoretical situation for a 100 ml blood sample immediately after a bolus injection of 25 mg of drug X before any partitioning. The blood and plasma are fully separated. This setup provides for an unaffected initial plasma drug concentration  $C_p$ .

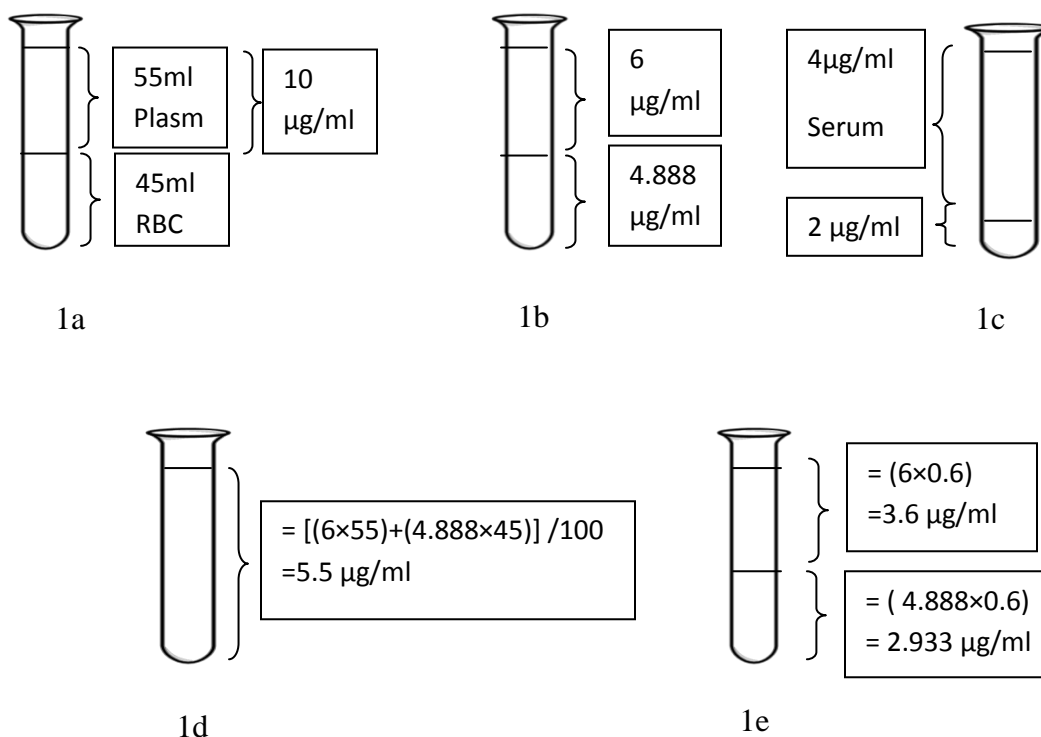
Figure 1b shows the changes in Figure 1a after the drug has partitioned in to erythrocytes. The concentration of the drug in erythrocytes ( $C_{ery}$ ) and in plasma water ( $C_p$ ) can be determined.

Figure 1c shows a clear 100 ml plasma sample collected from supernatant after centrifugation, which had an initial drug concentration of  $6 \mu\text{g/ml}$  as in Figure 1b. A fraction of drug is bound to plasma protein. The separated serum now has a lower drug concentration of  $4 \mu\text{g/ml}$ . Here serum drug concentration  $C_s$  can be determined.

Figure 1d is similar to figure 1b but with lysed erythrocytes releasing the drug throughout the 100 ml sample. This gives the whole blood drug concentration  $C_b$  calculated as follows.

Total drug in Figure 1b is calculated as follows. The amount of drug in plasma fraction =  $6 \mu\text{g/ml} \times 55 \text{ ml plasma} = 330 \mu\text{g}$ . The amount in erythrocyte fraction =  $4.888 \times 45 = 220 \mu\text{g}$ . Therefore the total drug in Figure 1d following erythrocyte lysis is  $330 + 220 = 550 \mu\text{g}$ . The  $550 \mu\text{g}$  quantity of the drug is distributed throughout the 100 ml sample. Therefore the resulting  $C_b$  is  $550 \mu\text{g}/100 \text{ ml} = 5.5 \mu\text{g/ml}$ .

Figure 1e is similar to Figure 1b but assumed that 40% of the drug has diffused in to tissues.



**Figure 1:** Partitioning of 25mg IV bolus. 1a; Drug in plasma immediately after injection. 1b; A fraction of drug partitioned in to erythrocytes. 1c; Precipitation of plasma proteins with bound drug from plasma fraction in 1b. 1d; Lysed erythrocytes releasing drug. 1e; Drug partitioning as in 1b when 40% drug has diffused in to tissues.

**Demonstration of aberrations in  $V_D$  values using model calculations:**

According to Figures 1a – d several drug concentration factors can be identified in different samples of same blood. They are  $C_p$  6 µg/ml (Fig. 1b),  $C_{ery}$  4.888 µg/ml (Fig. 1b),  $C_s$  4 µg/ml (Fig.1c),  $C_{protein}$  2 µg/ml (Fig.1c) and  $C_b$  5.5 µg/ml (Fig. 1d). In understanding  $V_D$  studies properly blood

should be considered to have several sub-compartments<sup>6</sup>.

According to Figure 1b applying  $C_p$ ,  $V_D = (25 \times 1000)/6 = 4167$  ml. In Figure 1c applying  $C_s$ ,  $V_D = (25 \times 1000)/4 = 6250$  ml. In Figure 1d applying  $C_b$ ,  $V_D = (25 \times 1000)/5.5 = 4545$  ml. It is proposed that the correct  $V_D$  is 4545 ml based on  $C_b$  in Figure 1d. The difference between the highest and

the lowest  $V_D$  values amount to 6250 – 4167 = 2083 ml or 2.1 L.

**Theoretical demonstration of anomalies of  $C_P$  based  $V_D$  determinations when  $C_{ery}$  is neglected:**

An IV dose of 250 mg results in a 100  $\mu\text{g/ml}$  ( $C_P$ ) as explained earlier under subsection

‘**Model theoretical  $V_D$ .....**’. Take an extreme example where 98 $\mu\text{g/ml}$  of a drug has partitioned in to erythrocytes ( $C_{ery}$ ) and only 2 $\mu\text{g/ml}$  is left in plasma ( $C_P$ ) and also the reverse situation where 2 $\mu\text{g/ml}$  for  $C_{ery}$  and 98 $\mu\text{g/ml}$  for  $C_P$ . The  $V_D$  calculations based on the above concentration values will be as follows.

The  $V_D$  based on 2 $\mu\text{g/ml}$  ( $C_P$ ):  $V_D = (250 \times 1000)/2 = 125000 \text{ ml}$  or 125 L. (Eqn. A)

The  $V_D$  based on 98 $\mu\text{g/ml}$  ( $C_P$ ):  $V_D = (250 \times 1000)/98 = 2551 \text{ ml}$  or 2.55 L. (Eqn. B)

Whole blood drug concentration  $C_b$  can be calculated as shown below based on Figure 1d.

For the first set of data,

Amount of drug in plasma = 2 $\mu\text{g/ml}$  X 55 ml = 110  $\mu\text{g}$

Amount of drug in erythrocytes = 98 $\mu\text{g/ml}$  X 45 ml = 4410  $\mu\text{g}$

Total drug in 100 ml = 4520 $\mu\text{g}$

Therefore  $C_b = 45.2\mu\text{g/ml}$  and the  $V_D$  based on  $C_b = (250 \times 1000)/ 45.2$  or 5.531L (Eqn. C)

For the second set of reversed data:

Amount of drug in plasma = 98 $\mu\text{g/ml}$  X 55 ml = 5390  $\mu\text{g}$

Amount of drug in erythrocytes = 2  $\mu\text{g/ml}$  X 45 ml = 90  $\mu\text{g}$

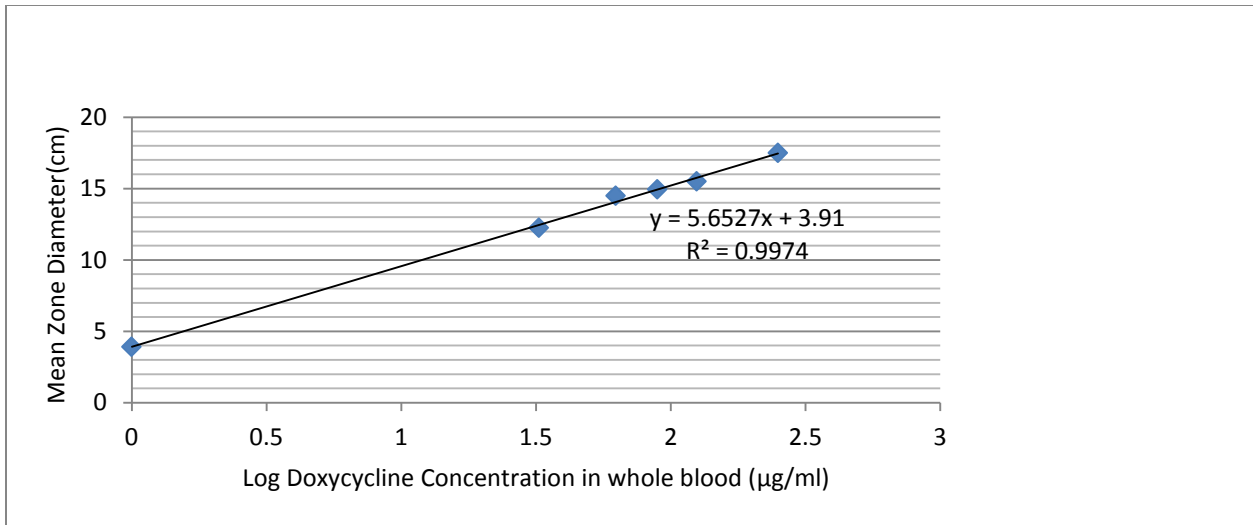
Total drug in 100 ml = 5480 $\mu\text{g}$

Therefore  $C_b = 54.8\mu\text{g/ml}$  and the  $V_D$  based on  $C_b = (250 \times 1000)/ 54.8$  or 4.562L (Eqn. D)

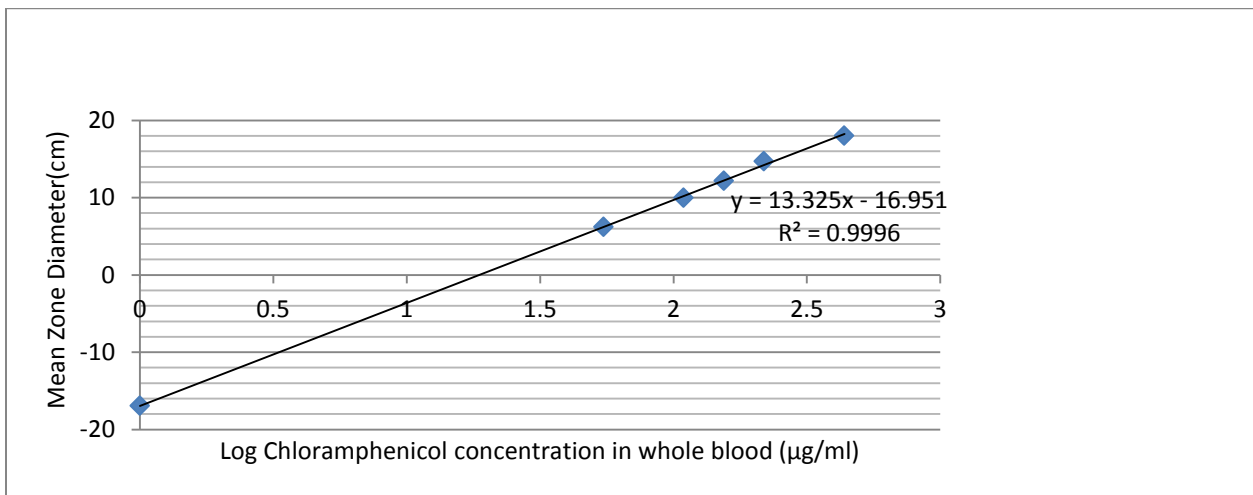
When considering 40% tissue drug diffusion, the values for plasma and erythrocytes can be expressed as  $C_P \times 0.6$  ( $\mu\text{g/ml}$ ) and  $C_{ery} \times 0.6$  ( $\mu\text{g/ml}$ ) respectively (Fig.1e).

Selected standard graphs for Doxycycline, Chloramphenicol and Rifampicin are shown

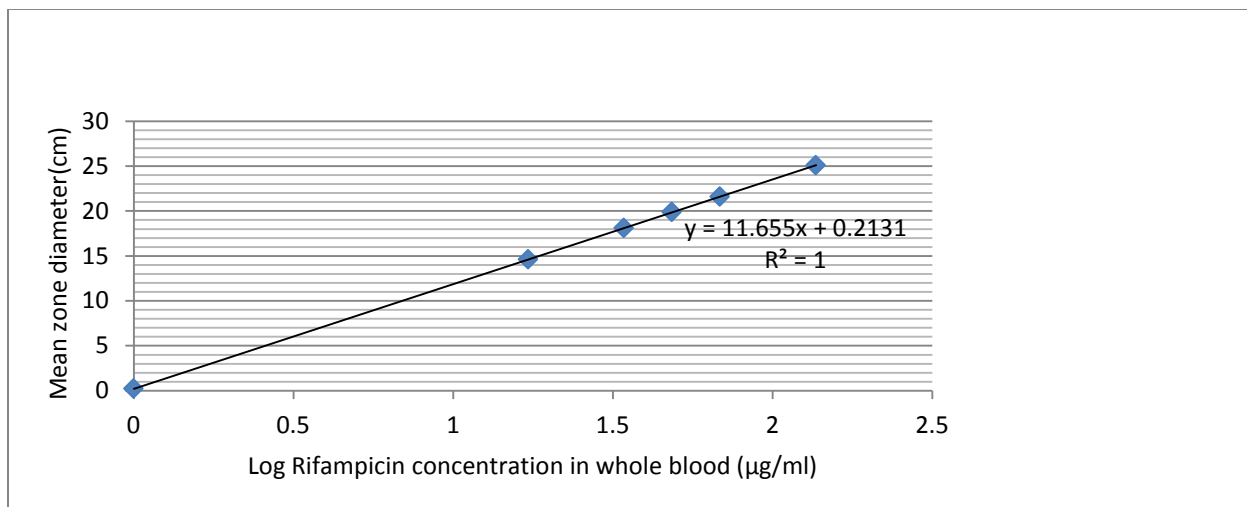
in Figures 2, 3 and 4. Zones of inhibition for supernatant and erythrocytes following incubation in drug solutions are shown in Figure 5. Drug concentrations partitioned in to erythrocytes and remained in the supernatant are shown in Table 1.



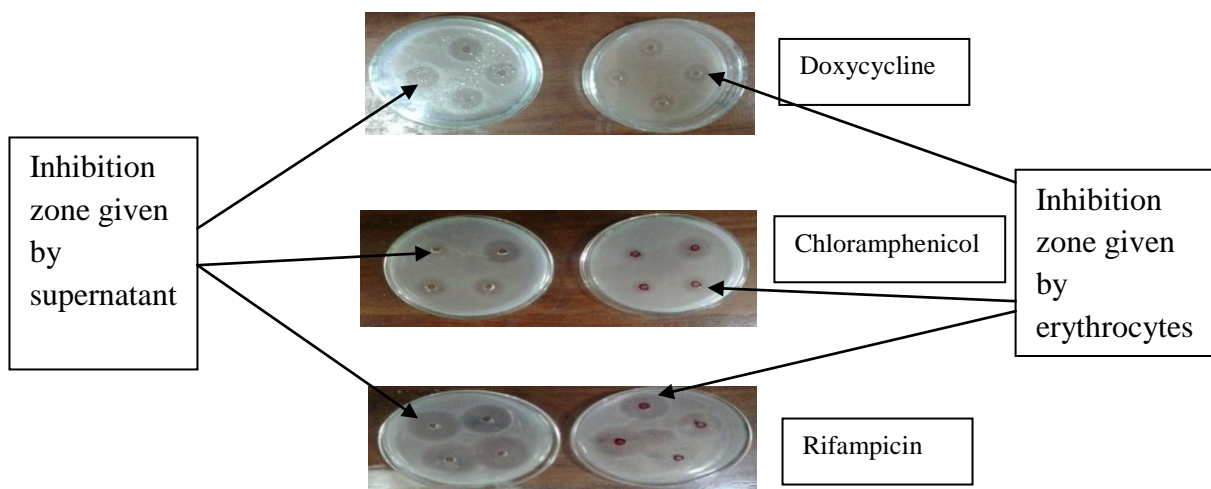
**Figure 2:** Standard curve for Doxycycline hydrochloride in whole blood.



**Figure 3:** Standard curve for Chloramphenicol in whole blood.



**Figure 4:** Standard curve for Rifampicin in whole blood



**Figure 5:** Inhibition zones given by erythrocytes and supernatant

### Results

Calculations for percentage difference of  $V_D$  values based on  $C_{Pw}$ ,  $C_{ery}$  and  $C_B$  given by the

third dilution for chloramphenicol,  $109.3\mu\text{g}/\text{ml}$  (Table 1) based on Figure 1b are shown below. Values are in same units for 40% tissue diffusion (remaining fraction 0.6) rare given in parenthesis.

$$\text{Drug in supernatant after partitioning (C}_{Pw}) = 49.3 \mu\text{g/ml (29.58)}$$

$$\text{Drug in erythrocytes after partitioning (C}_{ery}) = 56.8\mu\text{g/ml (34.08)}$$

$$\begin{aligned} \text{Drug in whole blood (C}_B) &= [(56.8 \times 2000) + (49.3 \times 2500)]/4500 \\ &= 52.63 \mu\text{g/ml (31.58)} \end{aligned}$$

$$\begin{aligned} \text{Total amount of drug in the body (D}_B) &= (109.3 \times 2.5 \times 1000) \mu\text{g} \\ &= 273\ 250 \mu\text{g (273\ 250, no change)} \end{aligned}$$

$$V_D \text{ based on plasma concentration} = (D_B / C_P) = (273\ 250 \mu\text{g} / 49.3 \mu\text{g/ml}) = 5543 \text{ ml} \quad (9238)$$

$$V_D \text{ based on erythrocyte concentration} = (D_B / C_{ery}) = (273\ 250 \mu\text{g} / 56.8 \mu\text{g/ml}) = 4811 \text{ ml} \quad (8018)$$

$$V_D \text{ based on whole blood concentration} = (D_B / C_B) = (273\ 250 \mu\text{g} / 52.63 \mu\text{g/ml}) = 5192 \text{ ml} \quad (8653)$$

$$\text{Maximum variance among three } V_{Ds} = 5543 \text{ ml} - 4811 \text{ ml} = \underline{733 \text{ ml (1020)}}$$

Drug in whole blood (C<sub>B</sub>) if C<sub>ery</sub> and C<sub>P</sub> values are reversed

$$= [(49.3 \times 2000) + (56.8 \times 2500)]/4500 = 53.47 \mu\text{g/ml (32.08)}$$

**Table 1:** Drug concentrations partitioned in to erythrocytes and remained in supernatant

Drug	Standard dilution series μg/ml	Plasma drug concentration		Erythrocyte drug concentration	
		C <sub>p</sub> μg/ml	%	C <sub>ery</sub> μg/ml	%
Doxycycline	250	233.18	93.27	30.78	12.31
	125	114.34	91.47	14.66	11.73
	62.5	61.11	95.74	4.32	6.85
	32.25	30.03	94.37	3.5	10.89
	437.1	210.7	48.2	222.2	50.84
Chloramphenicol	218.6	109.3	50.0	113.2	51.78
	109.3	49.3	45.11	56.8	51.97
	54.7	ND	ND	ND	ND
	136.9	74.1	54.13	63.4	46.31
Rifampicin	68.5	34.3	50.07	31.2	45.55
	34.3	20.5	59.77	15.8	46.06
	17.2	8.1	47.09	8.4	48.84
Oxytetracycline	50.0	21.26	42.52	28.24	56.48
	100.0	38.13	38.13	63.20	63.2
Chloroquine phosphate	8.0	4.56	57.0	3.44	43.0
	12.0	5.68	47.33	6.32	52.67

$V_D$  based on whole blood concentration =  $(D_B / C_B) = (273\ 250\ \mu\text{g} / 53.47\ \mu\text{g/ml}) = 5110\ \text{ml}$

No drastic difference in  $V_D$  values (5192 ml and 5110 ml) even when  $C_P$  and  $C_{ery}$  values are changed.

## Discussion

On an average adult blood volume of 4.5 L the erythrocytes occupy 45% equivalent to 2.0 L. The drug in erythrocytes is not represented in the formula  $D_B = V_D \cdot C_P$ . Most studies avoid  $C_b$  determinations due to the presence of hemaglobin from lysed erythrocytes. The regular formula for volume of distribution determinations should be  $V_D = D_B / C_b$ . This formula is mainly applicable for an IV administered drug that follows one compartment model. Drug partitioned into erythrocytes practically acts like a portion that has been spilt out of the compartment model studies. In this event all the determinations that follow are flawed.

There is a problem with protein binding  $C_{protein}$  (Fig.1c). In the case of the anticoagulant warfarin, protein binding is as much as 99% of the drug. The  $V_D$  values using  $C_P$  yield unrealistic results unless the analytical procedure extracts the bound drug as well. The degree of saturation of binding sites, the intensity of binding forces, type of bonds, functional groups of amino acids involved, types of plasma proteins involved, the polarity of extracting solvent system can all affect the extraction of the drug from the protein complex as against the free drug.

Equations A and B show that although the same amount of drug remains within the vascular system a drastic difference of over 40 times in the  $V_D$  values when  $C_P$  values are used in the calculations. Equations C and D show that results from  $C_b$  values will remain nearly equal with only about 10% difference. Under C and D, the  $C_{ery}$  fraction which has not diffused in to tissues is accounted reflecting the amount of drug held within the vascular system.

For all intent  $V_D$  informs us how the drug is distributed in the body. Accordingly, a drug that accumulates in tissues draw most of the drug out of vascular system leading to a low  $C_P$  and a large  $V_D$  as per formula  $V_D = D_B / C_b$ . Both the fractions of drug represented in plasma  $C_P$  and in erythrocytes  $C_{rbc}$  are intravascular based. These two fractions do not account for the drug distributed in to tissues which the  $V_D$  intends to reflect. Now a low  $C_P$  and a large  $V_D$  as described above will still appear to hold good even when  $C_{rbc}$  is five times the  $C_P$  as in the case of chloroquine infected with Plasmodia. It is a distortion of the real situation with respect to biopharmaceutical interpretation of disposition of a drug. There is not so much drug in tissues as it appears. In such a situation the use of whole blood drug concentration  $C_b$  which is a larger value than  $C_P$  will better reflect a comparatively lower  $V_D$  as most of the drug is accumulated in the erythrocytes.

The accurate formula for volume of distribution determinations should be  $V_D = D_B / C_b$  in which  $C_b$  accounts for drug in plasma, plasma protein bound drug and the



drug in erythrocytes<sup>7</sup>. In other words all of  $C_p$ ,  $C_{\text{protein}}$  and  $C_{\text{rbc}}$  should be lumped together using the weighted average as their volumes are not equal.

## Conclusion

The amount of drug partitioned in to erythrocytes and remained in plasma varied between large percentages as indicated in Table 1. There appear to be an increasing trend in the erythrocyte partitioning when the concentration of the drug was increased. Chloramphenicol, Rifampicin and Oxytetracycline shows significant amount of erythrocyte partitioning. It indicates a potentially deleterious effect on volume of distribution determinations if  $C_{\text{ery}}$  values are ignored. When  $C_b$  values are used no such differences occur as demonstrated by equations C and D. The determination of volume of distribution using only the plasma drug concentration could be misleading. The erythrocyte partitioning should to be taken into consideration to arrive at  $C_b$  for realistic  $V_D$  determinations.

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