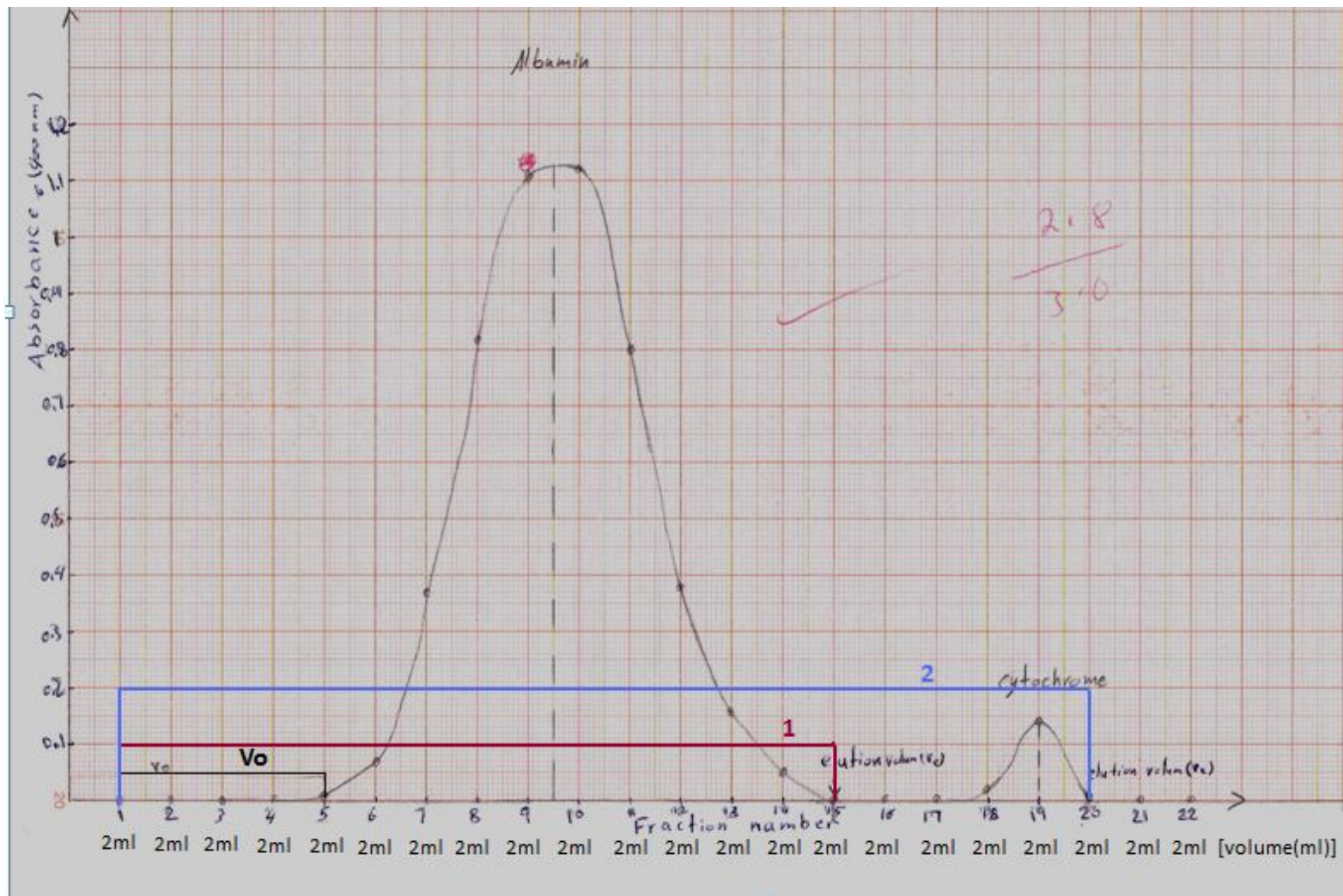


Gel filtration chromatography



Example:

Mixture of two proteins Albumin and Cytochrome having molecular weight [67.000 and 12.400] respectively , they were separated by using size exclusion chromatography. The collected fraction volumes was 2 ml each. The graph is represent the absorbance of the fractions collected from the column at 400 nm.

-The comment is in the next page:

As we can see from the graph there is two well separated peaks for a mixture of two proteins [Albumin and cytochrome] .

The first peak will be albumin protein. Because of its molecular weight [67.000, which is higher than cytochrome]. The long height of the first peak is the result of the high concentration of the protein [albumin] present in the sample mixture.

The first peak [albumin] begin from fraction [No.5] to [No.15]. So albumin was completely eluted from the column at fraction No.15.

The second protein [cytochrome] peak: will begin from fraction [No.18] to [No.20]. cytochrome was slowly in its flow through the column because it has small molecular weight [12.400] if we compare it with albumin M wt.

The lower height of the second peak is the result of low concentration of the protein [cytochrome] in the sample mixture. So, it will elute completely at fraction [No.20].

Note:

The void volume, V_o : the volume external to the beads.

The total volume, V_t : can be calculated from the dimension of the column.

The elution volume, V_e : the volume required for completely eluting the solute from column.

So, from the graph: [to calculate the elution volume]:

-**[1]:** 2 ml X 15 [fractions] = 30 ml of 0.1M NaCl was required to completely elute the albumin.

-**[2]:** 2 ml X 20 [fractions] = 40 ml of 0.1M NaCl was required to completely elute the cytochrome.

- **the void volume , V_o :** 2 ml X 5 = 10 ml. [the volume of liquid collected from the minute that the sample is applied to the column until an very high molecules which are unretained is eluted from the column].