From Fig. 10.3. we may have the following *two* expressions:

$$\text{Solvent Efficiency } [\alpha] = \frac{X_2}{X_1} = \frac{K_2}{K_1}$$

$$\text{Separation Factor } [SF] = X_2/X_1$$

Further more, it has been duly observed that:

- **α** Remains constant over a limited range of temperature, and
- **K** gets decreased with the increasing temperature.

i.e., both **'α'** and **'K'** happen to be solely **temperature dependent.**

Therefore, such a situation would ultimately lead to *decreased elution time*, and markedly *decreased separation* as well due to the fact that the **'analyte'** shall obivously remain in the **'gaseous phase'** for a longer duration in comparison to the corresponding **'liquid phase'**, which is actually responsible for the ultimate separation. Hence, to accomplish certainly improved and better separations of the desired **'analytes'** one must always use rather lower ranges of temperature, which would critically lead to the following advantageous benefits:

- increased liquid phase interaction,
- enhanced extent of separation, and
- extended time for entire analysis.

Note: The 'analyte' must remain at least (minimum) 50% of the total duration (time) in the 'liquid phase' in GLC.

[C] Resolution (R): In actual practice, the realistic and true separation of *two* consecutive peaks on a GLC **'chromatogram'** is precisely measured by resolution. Besides, it significantly provides the actual measure of the following *three* criteria, namely:

- column and solvent efficiencies,
- accounts for narrowness of peak, and
- critical observed separation between the maxima.

Figure: 10.4. illustrates explicitly the exact calculation of the resolution for the components duly present in the **'analyte sample'.**

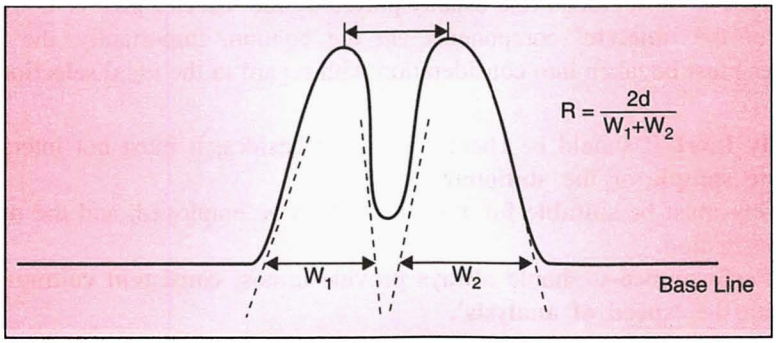

Fig. 10.4 : Calculation of Resolution between Two Peaks.

3.3.4.3. Support Coated Open Tubular Columns [SCOT - Columns]

The **support coated open tubular columns [SCOT - Columns]** are usually made by meticulously depositing **one micron thick porons layer** of an appropriate ***support material*** upon the ***inner walls*** of a **chromatographic capillary column,** and subsequently coating with a **thin film of** the **liquid phase.**

It is, however, pertinent to state here that the **SCOT-columns** do remarkably possess much higher degree of the **'sample capacity'**, and also give rise to definite **better resolution.**

Preparation of SCOT-Column: The **SCOT-Column** is invariably prepared by treating the **stationary phase** by carefully coating the **'liquid phase'** upon the absolute **inert stationary support media.** The necessary required coating is carried out by adopting the following steps in a sequential manner:

(1) Required amount of **'solid support material*** is weighed accurately, and transferred to a **rotary evaporation flask assembly.**

(2) **Liquid Loading** is duly accomplished by calculating and weighing the exact quantum of the **'Liquid phase'** on to the solid support media.

(3) **Liquid phase** is dissolved in a minimum quantity of the solvent so as to just wet the **'solid support media'** adequately.

(4) Gradually introduce the above **'dissolved liquid phase'** onto the **solid support material** in the flask of the **rotary evaporation assembly,** and affect constant uniform stirring until a perfect **'even slurry'** is duly achieved.

(5) Carefully attach the flask to a **rotary evaporator'**, and afford the solvent to evaporate completely. Thus, the slow rotation of the **'flask'** would ultimately ensure uniform coating of the liquid onto the surface of the **'solid support media'.**

(6) An appropriate chromatographic column is selected duly whose one end is adequately plugged with **glass wool;** and *via* the other open end the **prepared solid support material** [obtained from step (5)] is packed duly.

However, one may obtain perfect uniformly packed columns with the aid of the following *two* usually practised techniques for the **'coiled columns'** exclusively:

(a) **Electric vibrator** all along the length of the column, and

(b) **Constant gentle tapping*** in the course of slow addition

For **U-shaped chromatographic columns** the *'filling process'* is invariably done from either ends oriented towards the centre with electric vibrators.

(7) Finally, the duly packed column is placed in position into the **'column chamber of GLC',** which is subsequently **acclimatized** (or **conditioned**) by careful passage of the carrier gas (N_2) at 25 + 2°C above the operating temperature for a **fixed duration** or **24 hours.**

NOTE: A perfectly, and skilfully prepared gas chromatographic column should always maintain, and sustain an absolute zero base-line on the desired 'Chromatogram'.

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* Solid Support Material—of Knownand Correct mesh size

2. PRINCIPLES

In the past couple of decades, the **'classical liquid chromatography'** has been overwhelmingly supplanted by the more prominently adorable, reliable, and powerful analytical technique in the name of **high peformance liquid chromatography (HPLC).** There are *three* vital and important aspects that govern the underlying **principles of HPLC,** namely:

2.1. Stationary Phase Characteristics

The **stationary phase** materials invariably employed in **HPLC** do play a pivotal role in the critical performance, efficaciousness, and analytical profile for the assay of **drugs**. An ocean of knowledge, expertise, skill, and wisdom have been put together in the *design* and *development* of superb **HPLC-micro particulate column packing materials,** such as:*custom made silica* **particles** with remarkable **porosity, uniformity,** and **dimensions** varying between **3 to 10 µm** having either **spherical** or **irregular** shapes.

Examples: Such sophisticatedly architectured stationary phases may be used enormously for the critical separation of **nucleic acids, proteins, amino acids, antibiotics, steroidal drugs,** and a plethora of other **life-saving drugs, intermediates, metabolites,** and **biochemicals.**

2.2. Bonded Phase Supports

The latest most commonly used **packing materials** for the following *two* different types of **chromatographic** applications are, namely:

- **Silica Gel:** used for **straight-phase chromatographic** applications, and
- **ODS* Silica Gel:** employed for **reverse-phase chromatographic** applications.

However, it is pertinent to mention here that there exists a vast range of both **straight and reverse-phase packing materials** that are solely based upon meticulously designed **chemical modifications** affected upon the **surface of silica-gel;** besides, there prevails in recent years a latest trend to make use of *stationary phases* based on the so called **'organic polymers'**.

2.3. Retention *Vs* Polarity

It may be observed critically that the usual ensuing degree to which a **'drug substance'** executes the ability of ***retention*** shall solely depend upon the **drug's** inherent **polarity.**

Examples: The *two* typical examples are as stated under:

- **Silica gel:** ***polarity of compounds (drug),*** *and*
- **ODS-silica gel:** ***lipophilicity of compound (drug).***

Note: Importantly, most 'drug molecules' that have gained approval for usage by US-FDA essentially exhibit both 'lipoplilicity' and 'polarity'.

** **ODS:** Octa-decyl silane.

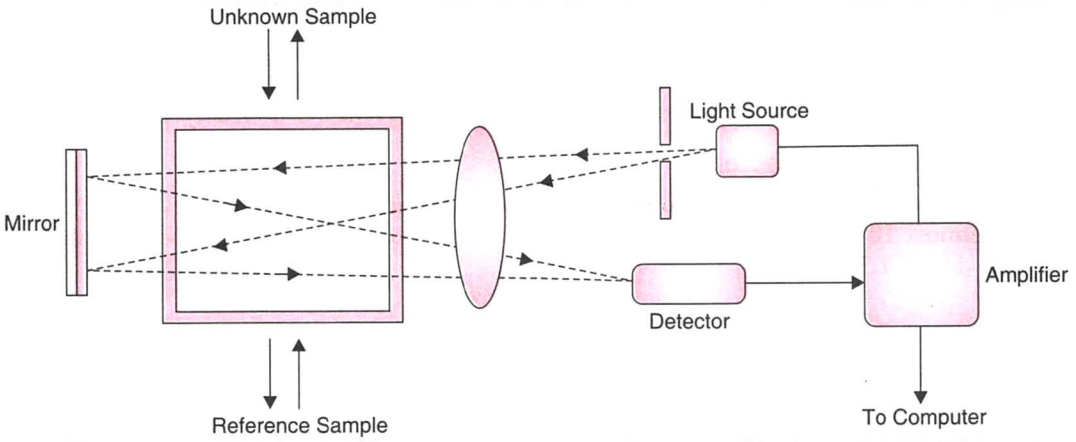

Fig. 11.8: Schematic Diagram of a Differential Refractive Index Detector

Salient Features These essentially include:

1. **Mobile phase** after elution from HPLC-column show its **RI** that varies depending on the exact and precise **presence** and **concentration** of *each component* in the **analyte sample.**
2. Eventually, **RI** of the eluent is differentially monitored *vis-a-vis* to a particular quantum of the **'mobile phase'** that has not yet crossed over *via* the **HPLC column.**
3. **Eluent** is made to pass *via* an optically transparent **flow-cell,** as the **blank (pure) mobile phase** moves across another **flow-cell**.
4. An **incident beam of light** is made to pass *via* both the cells individually (*i.e.,* **Reference Sample** and **Unknown Sample**); and thus, the **'detector'** placed strategically *monitors* and *regulates* the deflection of the emanated beams. Ultimately, one may record the **refractive indices** of the *two* **distinct solutions.**
5. A **'chromatograph'** is duly obtained due to:
   - close monitoring of **RIs** of **mobile phase** and corresponding **elute**, and
   - respective alterations in the **recorded RIs.**

Merits: These include:

- Majority of **'analytes'** may be **monitored** by using/adopting this specific approach, and
- **Slow-flow rates** shall not affect the **'sensitivity profile'** of this particular methodology.

Note: Differential RI detector techninque is observed to be highly sensitive to the fluctuations in temperature of the HPLC column even to the extent of 1/100th of a Kelvin.

Demerits: In fact, the **'demerits'** out number the *merits,* such as:

- **Differential RI detectors** may not be feasible in **specific HPLC protocols** which essentially engage **'solvent gradient elution'**, as it would certainly alter the observed **RI** of the ensuing **mobile phase,**
- Extent of achievable **sensitivity levels** may not closely match *vis-a-vis* to other **detectors** *viz.,*
  - **IR-detectors,** and
  - **Electrochemical detectors,** and