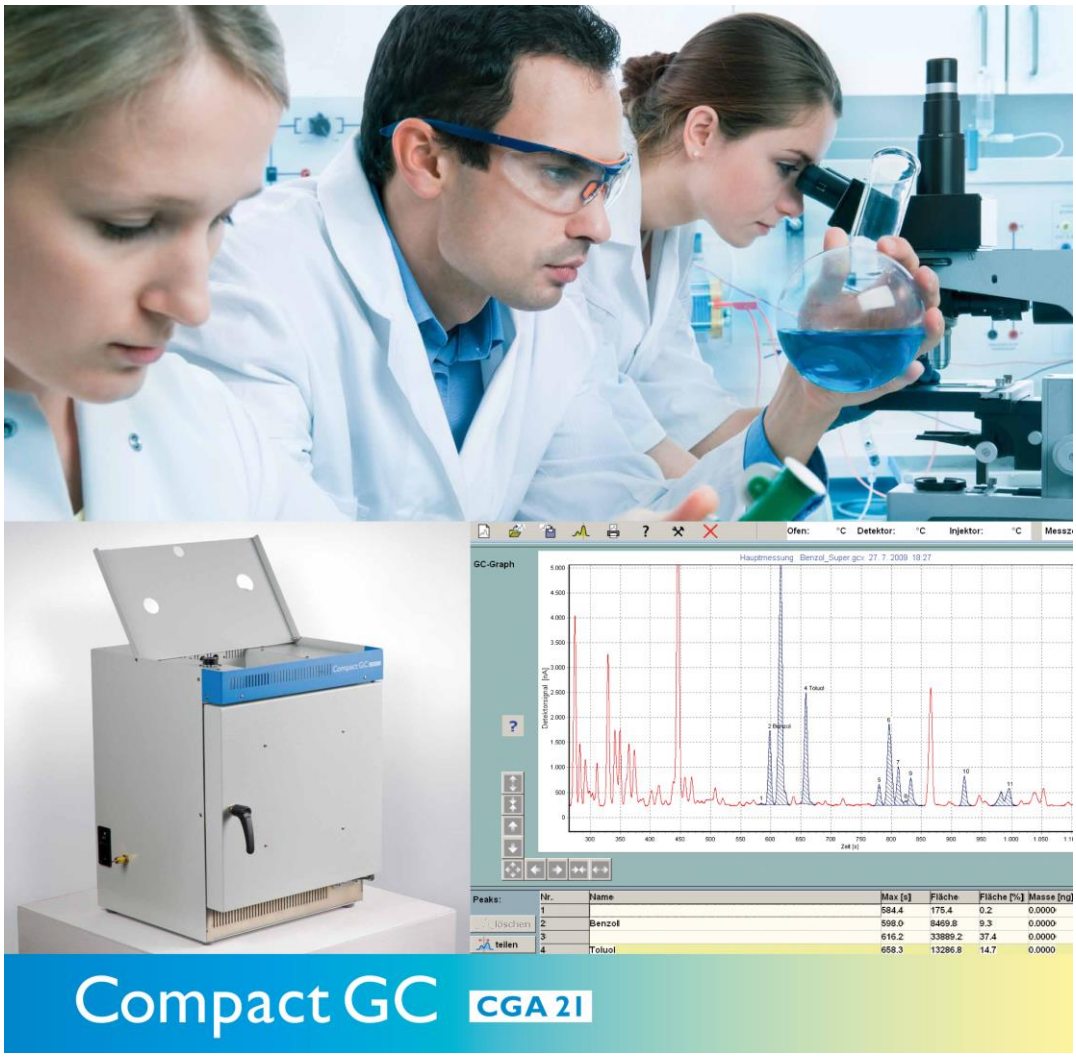
Gas-Chromatographs **CGA21**



User Manual

Version 3.12

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Contents

Chapter 1: Putting the device into operation

1.1 Safety considerations

Because this device is heated, it requires installation in an unobstructed area and must not be positioned next to highly flammable objects. The fan outlet on the right side and the air vents on the front side of the device must remain free to allow sufficient cooling of the device and the integrated electronics.

1.2 Mounting the gas flow bubble meter

Unpack accessories of the flow meter pack, it should contain: Flow meter tube, rubber bulb, mounting brackets, silicon tubing, screws M3

- Two threaded holes are provided on the left hand side of the chromatograph. Fasten the mounting brackets with two screws M3, horizontal part of the bracket at top
- Push the flow meter tube through the upper mounting bracket from the bottom up, then through the lower bracket from above.
- Fill the rubber bulb with soap solution (20 ml water and a drop of liquid detergent)
- Push the rubber bulb over the lower end of the flowmeter tube, wetting the glass end will help.
- Attach rubber tubing to the sidearm of the Bubble Flowmeter. The silicon tubing is then fed through a hole into the upper part of the GC housing.

1.3 Electrical connections

Power supply:

This device requires the usual mains supply voltage of 220-240V A.C. Viewed from the front, the supply voltage is fed in on the left by means of the accompanying connection cable for cold equipment. The main switch and fuse are also integrated in this terminal.

Connecting the control elements:

A standard PC connector panel is located on the right side of the device. All external devices (VGA monitor, USB flash drive, printer, keyboard and mouse) must be connected before the device can be operated. The plug of the monitor should be screwed tight to prevent loose contacts and increase operational reliability. Mouse and keyboard must be plugged into their corresponding colored connectors.

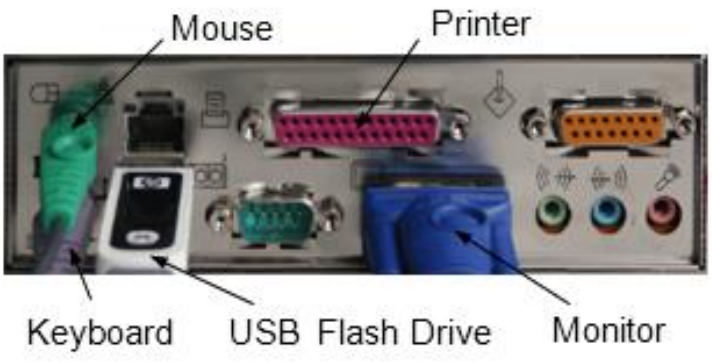

Fig. 1 Connector panel

1.4 Gas connection for TCD units

The carrier-gas connection for TCD units is also located on the left side of the device. We recommend using helium as the carrier gas for TCD units, as it is non-hazardous and ensures a sufficiently high sensitivity of the detector. The pressure reducer of the gas pump is connected using the components in the accompanying set of accessories. Connect one end of the hose from this set of accessories to the gas pump and the other end to the gas chromatograph. Hand-screw the union nut initially, and then tighten it gently with the spanner. Do not tighten the nuts too much! The gas pump is to be equipped with two-stage pressure reduction valve. Connections on the pressure reduction valves are not standardised, although the adapter supplied by us fits most valves. First attach the Teflon line with 1/8" tube adapter included in the connection set to the chromatograph. Hand-screw the union nut initially, and then tighten it gently with the spanner.

We recommend using helium with a purity grade of 5.0 (99.999%) for the device.

Larger quantities of oxygen in the carrier gas damage the separation column and the filaments in the detector.
Turn on carrier gas prior to operating the device!

1.5 Gas connection for FID units

Gas chromatographs with a flame ionisation detector principally need to be operated with three different types of gas:

1. The carrier gas
2. Hydrogene for the FID
3. Purified air (free of hydrocarbons) for the FID

One important advantage of the GC-CGA-3 compared to other devices is that it generates the purified air itself. Consequently it only has two connections for the hydrogen and carrier gas respectively. If hydrogen is also used as the carrier gas, then only one type of gas is required. In this case, the two connections are linked with a T-piece included in the scope of supply. The gas connection leads as well as the adapters for the hydrogen and carrier gas need to be ordered separately.

Two connection sets are required for operation with two gases. First attach the two Teflon lines with 1/8" tube adapters included in the connection sets to the chromatograph. Hand-screw the union nut initially, and then tighten it gently with the spanner. Then screw the transition pieces to the pressure reduction valves. As these transition pieces are not standardised, no matching spanner has been included in the scope of delivery. Tighten these screw connections firmly. Then attach the 1/8" tube connections to the transition pieces. Ensure that the hydrogen lines are connected to the terminals designated H2.

If the device is to be operated using only one type of gas, screw the accompanying T-piece to both gas inlets on the device and establish the connection with the hydrogen cylinder. In this case, only one connection set is required for hydrogen.

- **Only use suitable pressure reduction valves with a low output pressure**
- **Hydrogen must only be handled by qualified personnel.**
- **If hydrogen is used as the carrier gas: Check the column connections regularly and tighten if necessary.**

Chapter 2: Operating the device

The system software of the gas chromatographs CGA21 is based on Windows® . To a large extent self-explanatory, its context sensitive help system allows the device to be operated without using the written documentation a lot. However, basic knowledge of the principles of gas chromatography is indispensible as well as observance of the generally accepted safety rules, in particular if hydrogen is used when operating the FID.

2.1 Device messages during power-on

After turning on the device, different power on messages may appear.

2.1.1 TCD-Device: Warning: Turn on carrier gas

In the heated state, TCD units can be damaged even by minute traces of oxygen. For TCD units to provide trouble-free operation, you must therefore ensure that the supply of carrier gas is sufficient at all times (refer to "Summary of the essential properties of a TCD:") If a TCD unit is turned on, it will issue a corresponding warning message. It is only after you have acknowledged this message with OK that the heating of the TCD is activated; in this manner, you can ensure a sufficient supply of carrier gas without causing any damage to the detector.

2.1.2 FID-Device: Advice: Measuring gas flow at start up

As a rule, the flame ionisation detector is heated to its minimum temperature of 150°C immediately after the device has been activated, to prevent water from condensing in the detector. At this high temperature, it is no longer possible to measure gas flows; for this reason, the message displayed on the right is issued immediately after the GC has been turned on. If you then select „Measure", the detector remains unheated until the first measurement has been started. This allows you to dismantle the unheated detector and measure the gas flows first. If you select OK instead, the initial menu of the device is called up. Measuring gas flow is described in chapter 6.

2.2 Main window

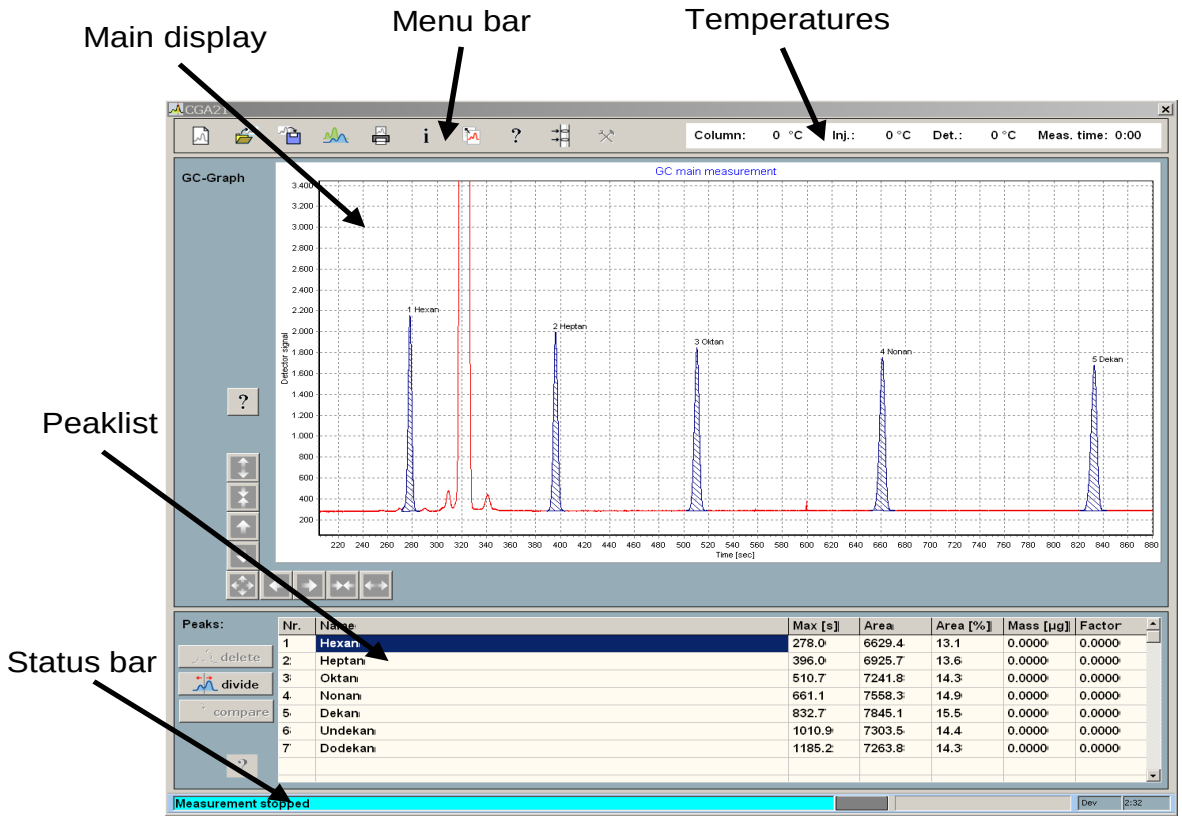

Fig. 2: Main window

Functional components of the main window:

- Menu bar with temperatures
- Main display
- Peaklist
- Status bar

2.2.1 Menu bar

The menu bar buttons trigger basic actions. Rollover with mouse cursor pops up hints.

New Measurement: see chapter „New measurement“

Open measurement: Opens existing measurement. Supports older binary *.gcd files as well as the current XML format *.gcx.

Save measurement: Stores existing measurement in XML file format (file extension: .gcx) In general it is recommended to store measurements on USB flash disk and not on the system disk.

Comparison of chromatograms: Allows you to compare a chromatogram with one or more reference chromatograms. For details see chapter 2.4 “Evaluation of a chromatogram“. **This button toggles between two states.**

Print report: Print report as pdf–file. The report includes the temperature program, user data, main display with currently selected section and the peak list. Although possible, we do not recommend using a printer with a CGA21. It is better practice to take measurement files to a PC workstation on an USB flash drive and make printouts there. As an alternative you can make pdf files at the GC, save it to USB flash disk and make printouts at a PC workstation.

Information about the current measurement: Displays information about the open measurement, such as temperature program, user data etc.

Save main display to file: Saves an image of the main display into a emf – file. This allows for convenient insertion of GC curves into every common office program.

Help: Access to common help. Important windows contain additional context sensitive help buttons.

Measuring gas flows: Utility for convenient measurement of gas flows using the soap bubble meter.

Options: Reserved for future use

2.2.2 The main display

The display allows for easy visualization and evaluation of GC measurements. Use the „Open Measurement“ dialog in the usual manner. The chromatogram will be scaled automatically to fit the display area.

Symbol colors:

Red: Regular GC curve

Blue: Peaks

Green: Peaks with a known mass are colored green. They can be used to determine the mass of peaks with unknown mass, provided it is the same compound.

Yellow: Indicates marked peaks. Marked peaks can be deleted using the „delete peak“ button.

Using the main display (GC Graph):

Pan: simply move the mouse while pressing down the **right** mouse button. Alternatively use the arrow buttons.

Zoom: Draw a rectangle from top-left to bottom-right while holding down the **left** mouse button. Mouse gesture in the opposite direction (bottom-down → top, left) restores standard view.

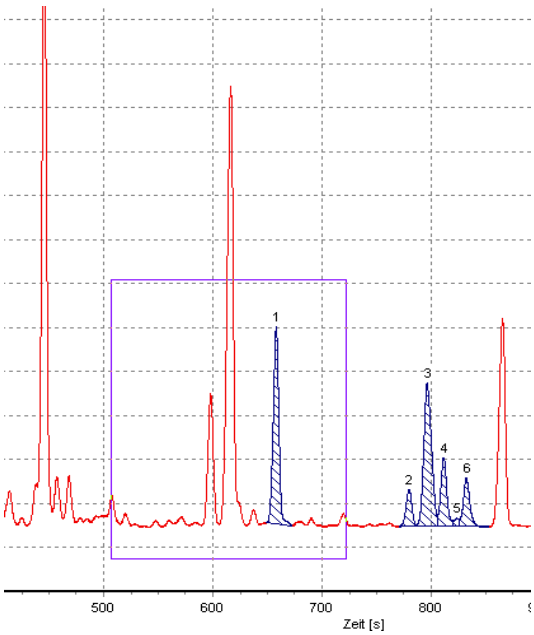

Fig. 3: Select zoom area

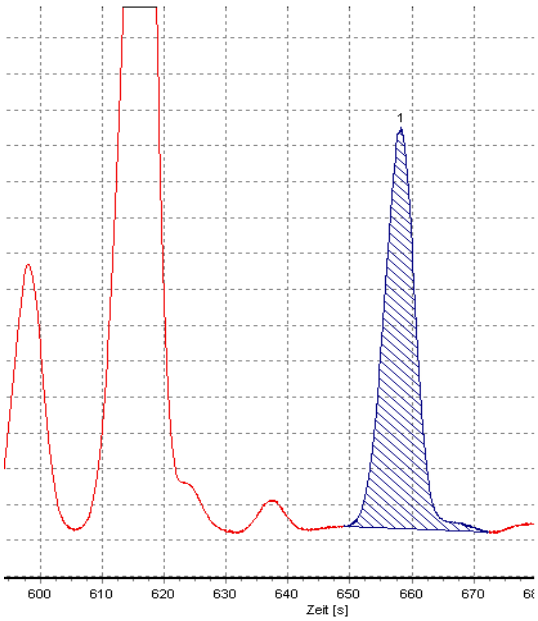

Fig. 4: Enlarged section

Actions:

Create a peak: Peaks can be created by double clicking into the display. The first double click creates a starting point which is indicated by an arrow labeled „start“, the second double click marks the endpoint of a peak. The second double click must be at higher time values than the first.

Mark peak: Double click into the (blue) peak: Peak will be marked and its color changes to yellow. More than one peak can be marked

Divide Peak: Peaks can be divided. Left click button „Divide peak“, the mouse cursor turns into a vertical line. The next left click divides the peak under the line cursor.

Delete peak: A left click onto button „delete peak“ will erase all marked peaks.

2.2.3 Peaklist

Marked peaks will be added to the peaklist immediately. The entries of the peaklist will be transferred into the measurement file when a „Save“ command is executed. Every line in the peak table represents one peak with its corresponding properties. Fig. 5 shows a mixture of alkanes as an example:

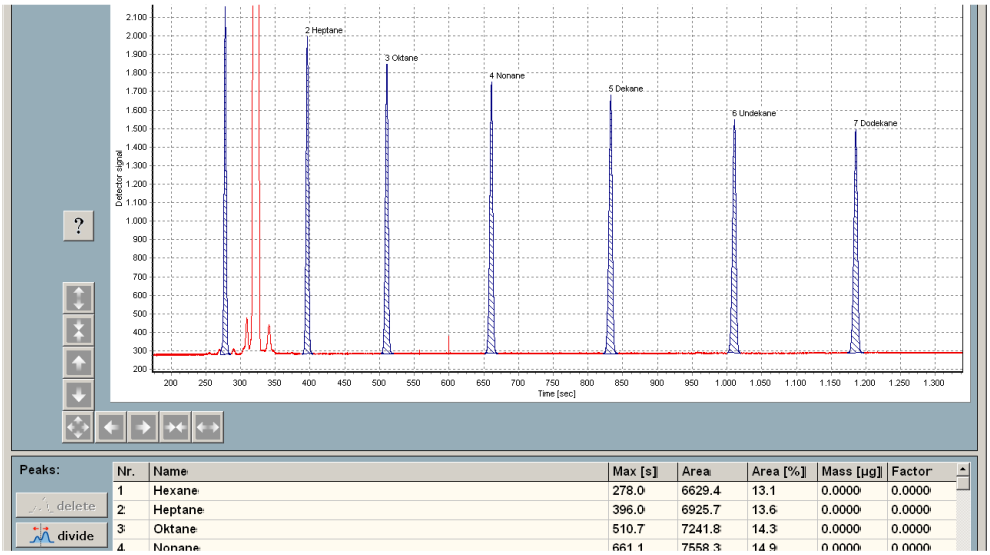

Fig. 5: Peaklist

The columns of the peak table:

Nr.: Peak number in ascending order of their retention times.

Name: Name of peak given by user. To assign a name to a peak, left click into the peak. The table now shows this peak in the first line highlighted in light yellow. After a double click into the name field a window pops up where the name can be entered.

Max[s]: Position of maximum: Elapsed time between injection and peak maximum. Time in seconds.

Area: The Peak area is proportional to mass for a given substance. It is determined automatically by numerical integration in arbitrary units. Please note: The relationship between mass and area of a peak is not trivial, it depends among others from the kind of detector used, the substance and the split ratio. As a consequence these values are only useful for comparison.

Area[%]: Percentage share of peak area in the sum of all marked peak areas. If only one peak is marked, this percentage is 100%.

Mass and factor: If the peak has been standardized with a reference measurement, this column shows the mass in [ng] and the factor for conversion of peak area into mass – *and vice versa*. These columns can be used in two different ways:

1. Let's assume a reference measurement has been made. In this case the substance and its mass is known. Therefore it is possible to enter the mass of the substance into the table, again by double clicking the mass field in the row of the respecting peak. After having entered the mass, the factor is determined automatically.

2. A measurement like the one mentioned above can be used to determine an unknown portion of the same substance in another measurement by comparing the two measurements via the menu button "measurement compare" This procedure is described in detail in chapter "2.4 Evaluation of a chromatogram" and in an example in chapter "3.3 Benzene in gasoline".

2.2.4 Status bar

Displays information about the state of the chromatograph: (from left to right)

- Textual information about the current state of the device
- TCD Thermal Conductivity Detector on/off
- Measurement progress
- Detector type
- Time elapsed since the GC has been turned on.

2.3 How to conduct a GC measurement

2.3.1 Preparations

- Check gas connections.
- TCD-Device: make sure carrier gas is turned on.
- FID-Device: ignite FID, detector must have reached a temperature of 150°C ! For details see chapter 2.3.4

2.3.2 Set measurement parameters

Clicking „New measurement“ shows the parameter window:

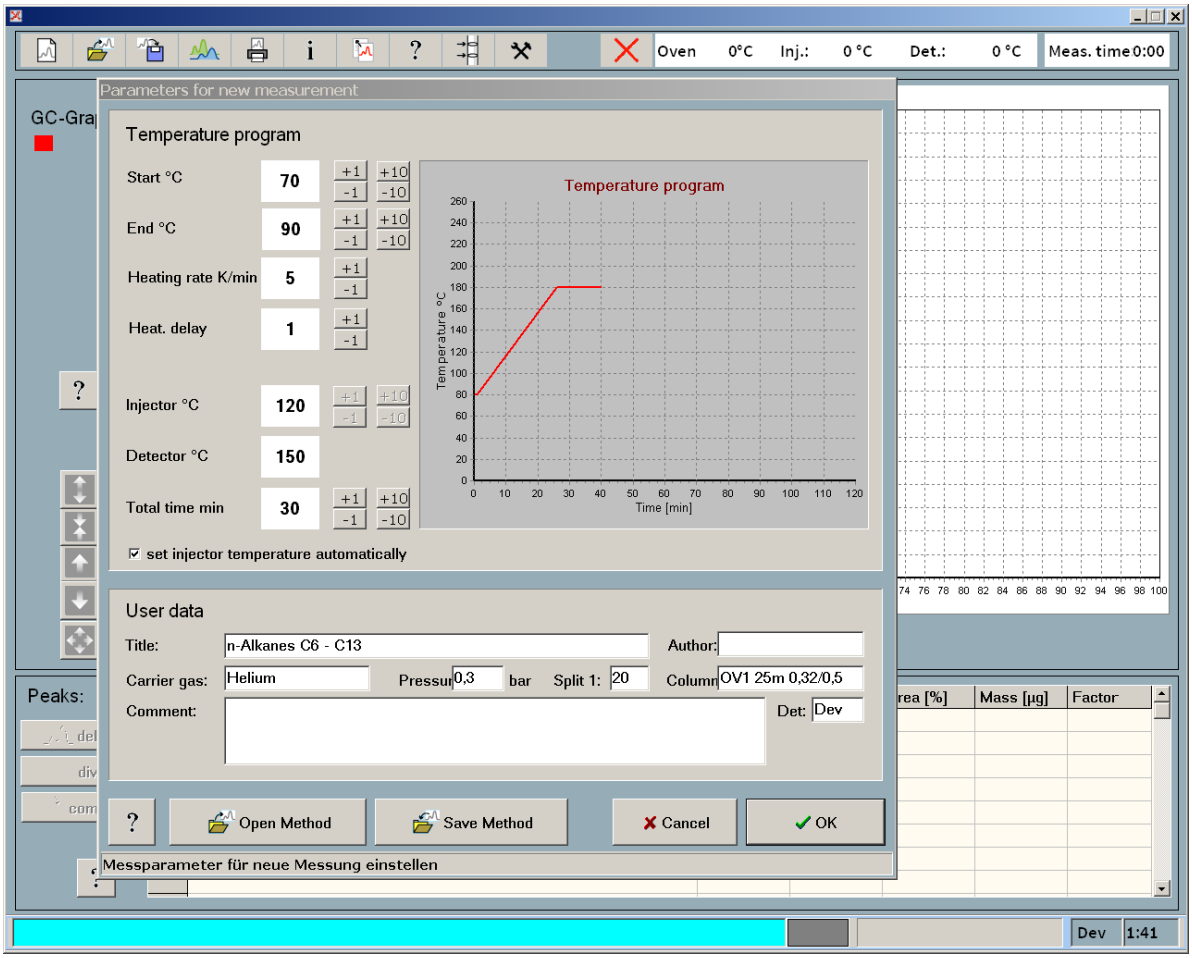

Fig. 6: Parameter window

The temperature program of the column furnace is set in the upper section of the window. Basically, two different types of measurement can be configured here:

- **Isothermal measurement**: The temperature of the separation column remains constant during the measurement.
- **Dynamic temperature measurement**: In many cases, the task on hand makes it necessary to progressively increase the column temperature during a measurement. A total of four parameters need to be entered in this context:

    1. The initial temperature at which the measurement is starting.
    2. The final temperature at the end of the heating phase.
    3. The start of the heating phase, indicating the length of the preceding, isothermal phase.
    4. The heating rate, i.e. the rate in degrees/minute at which the temperature is to rise during the heating phase.

The individual values of the temperature program can be set with the arrow keys next to each field. The entered values are checked for plausibility, and dependent values are **corrected accordingly**. For example, if the final temperature you have selected is lower than the initial temperature, then the initial temperature is lowered automatically to the same value as the final temperature

The minimum temperature of the detector is 150°C. At higher final temperatures, the detector is heated accordingly before commencement of the measurement. The temperature of the injector is 30°C higher than the selected final temperature. You can change this property by unchecking the “Determine Injector temperature automatically” checkbox. In this case, the detector temperature has to be entered manually. Click on “OK” to accept the displayed values, click on” *Cancel*” to abort preparations for the measurement.

The column furnace, detector and injector now need time to reach the set temperatures. Several different messages can appear now, depending on the instantaneous temperature of the furnace.

Input of user data

The lower section of this window contains several text fields where important information concerning the measurement can be entered. However, this information is optional and does not affect the measurement itself and can also be changed subsequently if required. The detector type is set by the system and cannot be changed

Loading and saving methods

By pushing the “Save method” button the contents of the parameter window can be stored into a method file (file extension .gcm) while the “Load method” button allows for restoring these values from file. In both cases the usual file dialog is used. It is advised to have a separate folder containing method files. Instructions for sample preparations can be integrated into the HTML – help system.

2.3.3 Preparations for measurement

Closing the parameter window with the „OK“ button initiates the start procedure of the

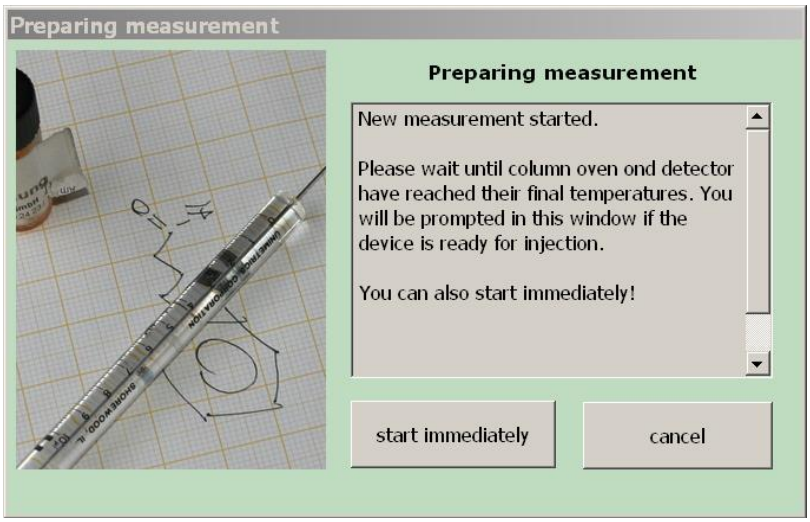

Fig. 7: Dialog prepare measurement

measurement. Before the actual measurement can take place, it is necessary for the column and the detector to reach their final temperatures. You will be notified about this in the same window. You can also start the measurement immediately, in this case, however, measurement is made with an invalid temperature program.

After the dialog has changed to the following message, all temperatures are valid.

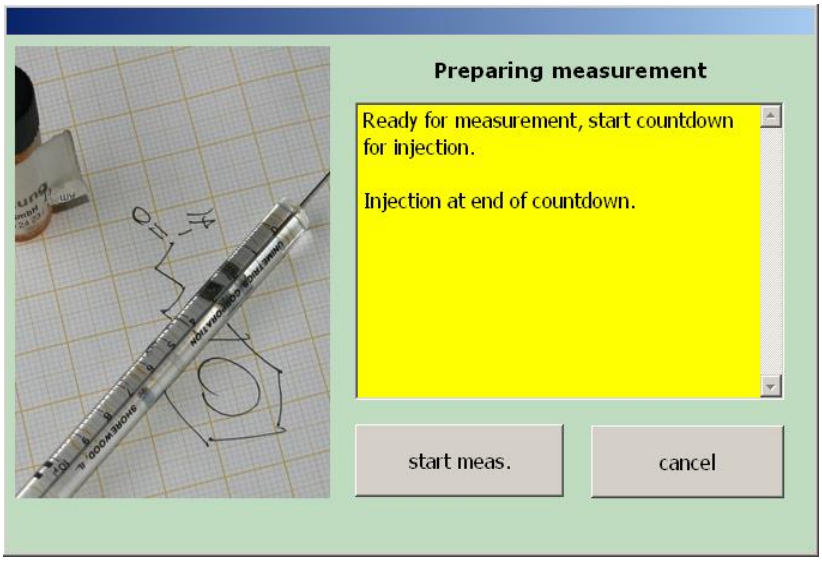

Fig. 8: Dialog ready for injection

Now it is time to fill the microliter syringe with the substance to be analysed and adjust the volume to the required value. Once all the preparations for the measurement have been completed, click on “*start meas.”*. In the picture then a number appears displaying a countdown sequence which gives you 10 seconds time to insert the needle of the syringe through the septum. Once the countdown has elapsed to zero, inject the test substance. Then withdraw the microliter syringe from the injector. If a high injector temperature has been

selected, it is advisable to insert the needle of the syringe as late as possible - depending on how practised you are - in order to prevent premature vaporisation of the test substance from the needle. In this context, also refer to the chapter *3.2 Notes on Injection*

The actual measurement procedure can now be commenced. The components of the test mixture drift at different rates through the separation column. Their emergence from the column is registered electronically and recorded at certain time intervals. At the same time, these values are plotted as a characteristic on the screen. The display of this characteristic can be adjusted during the measurement.

Without intervention by the operator, the device automatically terminates the measurement after the scheduled time. However, you can terminate the measurement manually at any time by clicking “Stop measurement”.

In the case of TCD units, it is also possible to shift the reference line with the buttons labelled “baseline up” and “baseline down”, e.g. to correct drifts caused by the detector. This function is not available for FID units, due to their negligible drift.

2.3.4 Ignition of the FID

The functional principle of the FID is based on ionisation of hydrocarbon fragments inside a hydrogen flame. Prior to any measurement the FID therefore has to be ignited. As the hydrogen flame is operated with an excess of combustion air, ignition is only successful if the excess of air is reduced for a short time. Ignition can be visualized because the detector current changes dramatically in the course of this process. Simply start a new measurement before ignition and take a closer look at the resulting curve, which is didactically interesting too:

Igniting the Flame Ionisation Detector

- Wait until the detector temperature is higher than 100 °C.
- Memorize (or better write down!) the position of the hydrogen valve.
- Turn the valve ¾ turn counter clockwise.
- Fan the flame of a gas lighter over the top of the FID. Ignition is indicated by a small oxyhydrogen explosion.
- **Don’t forget to turn back the hydrogen valve to its former position**.

2.4 Evaluating a Chromatogram

In first approximation the area of a chromatographic peak is proportional to its mass. Prerequisite for quantitative evaluation of peak masses is the knowledge of the proportional factor that joins area and mass of substance. This factor can be determined by calibration with a known amount of substance. A measurement containing peaks of known mass is termed **reference measurement** here and those peaks with a known mass are colored green in the display.

This means the determination of absolute masses in a chromatogram requires two measurements, the reference measurement *with a known* amount of substance and the main or test measurement containing an unknown amount of the same substance. It is mandatory, that these two measurements are conducted under the same conditions, the most important of which are: Split ratio, temperature program, gas type and pressure.

Way of proceeding:

- **Open** the main or test measurement by clicking the “Open measurement” button.
- **Mark** peak to be evaluated by double clicking the starting point and the end point of the peak. The area under the marked peak turns to blue.
- **Click** “Compare measurement**”** in the main menu. A second diagram shows up.
- **Open** the reference measurement by clicking the “Open reference” button. The reference chromatogram will appear in the upper diagram.
- **Pan and zoom both measuremts** in ordert to have both the reference peak (green) and the test peak approximately one above the other.
- **Click** „Compare peak“, located on the left hand side of the peak table .
- **Move the mouse cursor** into the main measurement diagram. The shape of the cursor changes into a dotted line that runs through both diagrams. Position the line in order to have it run through both peaks and click left.

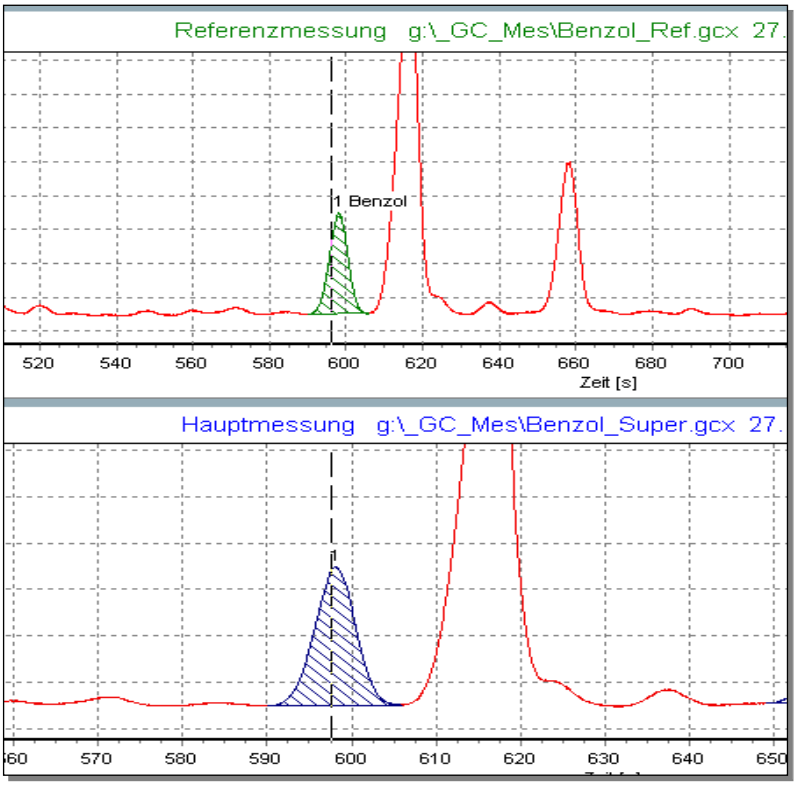

Fig. 9: Comparing peaks

Now the mass and the peak factor are shown in the peak list. The calibrated peak turns green as its mass is known now. It makes sense to give this peak a name, so double click the corresponding name cell and enter the name. Don't forget to save the changes!

Chapter 3: Examples

This section provides an example elucidating the handling and operation of the chromatograph. The fundamentals of sample introduction are explained first; followed by an example of analysing a hydrocarbon mixture and evaluating the resulting measurement. In this example, the benzene content in supergrade petrol is determined.

3.1 General note using this software

The examples can be comprehended with the CGA21-Software not only with the gas chromatograph itself, but also by means of simulation software on every Windows workstation in your lab. Of course you can't make real measurements with a PC, but evaluation of measurements as well as the single steps that lead to the actual measurement can be demonstrated in the lecture hall. The USB pen drive, that's included in delivery contains the system software of your CGA21 in portable form running on every Windows PC using Windows XP up to Windows 7 without any installation.

Here's how to use the pen drive with the CGA21 software:

- Plug the flash drive into an USB connector of your Windows PC.
- Open the Explorer (e.g. right click Start button, than select Explorer) If autorun comes in your way, cancel it.
- Search for the pen drive, its name is CGA21, navigate into folder _CGA21 and double click gc.exe
- Depending on your operating system you have to agree to security warnings

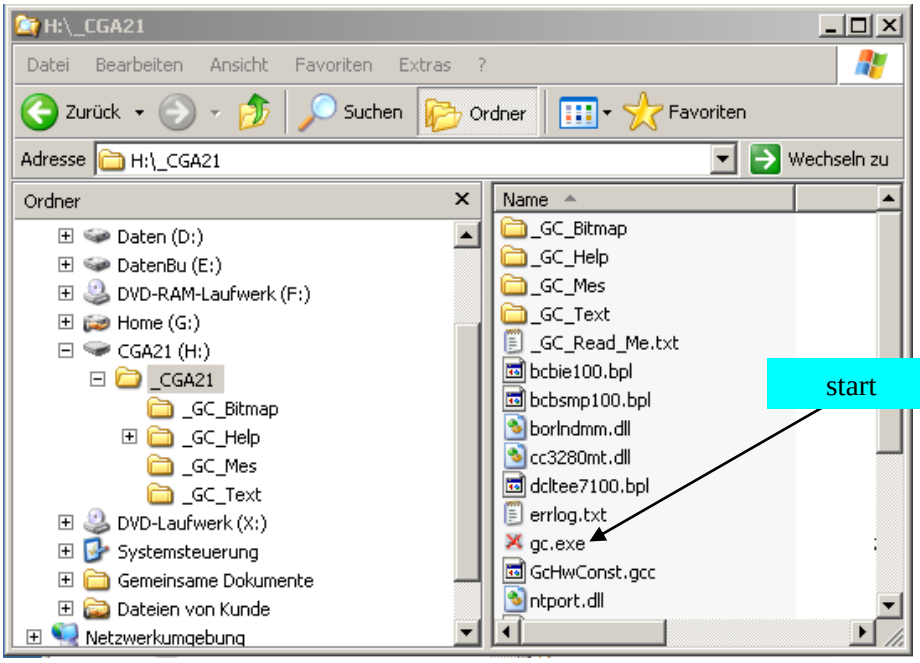

Fig. 10: Starting the software on a workstation

After the program has been started a GC measurement is loaded automatically so you can immediately start using the program. The software may be handed over to employees, students or pupils of your institution.

No software is perfect. Please help improving this program (or the manual)! Send bug reports or suggestions to *frank.beer@s-lg.de*

3.2 Notes on injection

Sample introduction is the only step in the operation of a chromatograph which requires a certain amount of skill and practice in order to achieve reproducible results.

To fill the syringe, push the plunger in to the limit, immerse the needle in the liquid, and then draw the plunger slowly to a point far beyond the required volume. Observe the column of liquid in the syringe. If it is largely free of bubbles - very small air bubbles with a volume of approximately 0.01µl cannot be avoided in practice - then push the plunger forward until the required volume is reached. Any air bubbles still remaining can be compensated. Example: The column of liquid contains an air bubble approx. 0.1µl in size and the required volume is 1µl; consequently, the plunger is set to the 1.1µl mark. If the column of liquid contains too much air, then "pump" the plunger briskly - but not too briskly - down to its limit, draw in liquid again slowly, and observe the liquid column. If the amount of air appears sufficiently small, then proceed as before and adjust the sample volume, allowing for the air quantity.

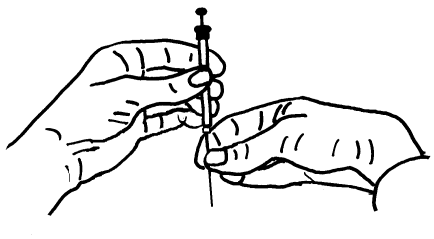

Fig.11: Inserting the needle into the injector

During injection, a compromise needs to be struck between two conflicting requirements. On the one hand, the injection must be rapid as the thin needle of the syringe heats up very quickly in the hot injector; if the sample in the needle starts boiling, part of the substance will be ejected prematurely from the syringe. This will result in widened, deformed or even duplicated peaks. On the other hand, the relatively thin and sensitive needle needs to penetrate the septum of the injector, an act which requires a certain amount of effort as well as caution. If you only hold the glass cylinder of the syringe, the needle will bend sideways and, in the worst case, become unusable. For this reason, you should use one hand to hold the glass cylinder and guide the needle with the thumb and index finger of your other hand. Once the needle has been fully inserted, press the plunger down quickly and then withdraw the syringe immediately. This procedure should be coordinated in such a way that the plunger is pushed down as soon as the countdown of the chromatograph has elapsed to zero. Details on starting a new measurement are provided in chapter 2.3

3.3 Determining the benzene content in supergrade petrol

In some countries it is not allowed to carry out experiments with Benzene in school labs. To comply with applicable regulations use other substances eg. Toluene instead of benzene.

3.3.1 Identifying a substance in a chromatogram

The investigation of mineral-oil products is an important domain in high-resolution gas chromatography, as these complex mixtures cannot be analysed so elegantly using any other method. The content of benzene in, present in varying quantities of up to a few percent in carburetor fuel, is to be measured in this example. The measurements are in directory *_CGA21_GC_Mes*

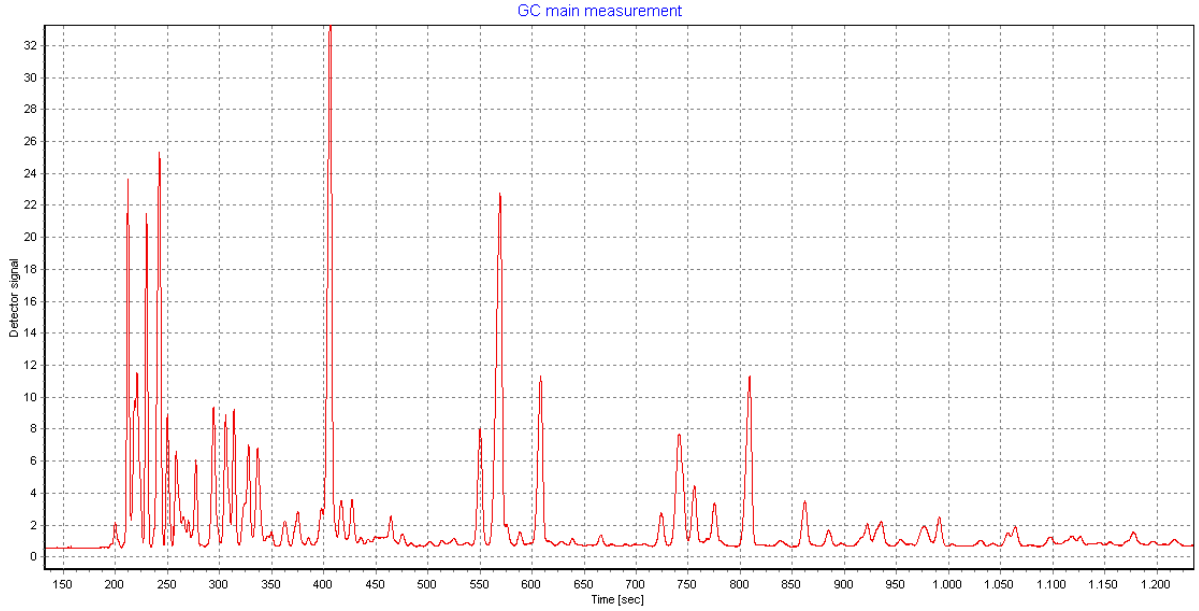

Fig. 12: Chromatogram of supergrade petrol

One of the peaks in the chromatogram above is benzene. Which one is it?

To identify a particular peak, add a certain amount of the substance you are searching for, i.e. benzene in this case, to the sample of the test mixture. The quantity of the added substance should be roughly equal to that already present in the mixture. In this example, 10% by volume of benzene has been added to the supergrade petrol. The sample is then analyzed with the same parameters as used for the original sample, and the two chromatograms are subsequently compared.

For comparison, click “Compare chromatogram” in the main menu and observe both the chromatograms on a suitable scale. If the test substance contains benzene, then the second chromatogram should contain a peak also present in the original one, but considerably smaller there. Fig. 13 shows the comparison identifying the benzene peak, which is much larger in the upper chromatogram than in the lower one.

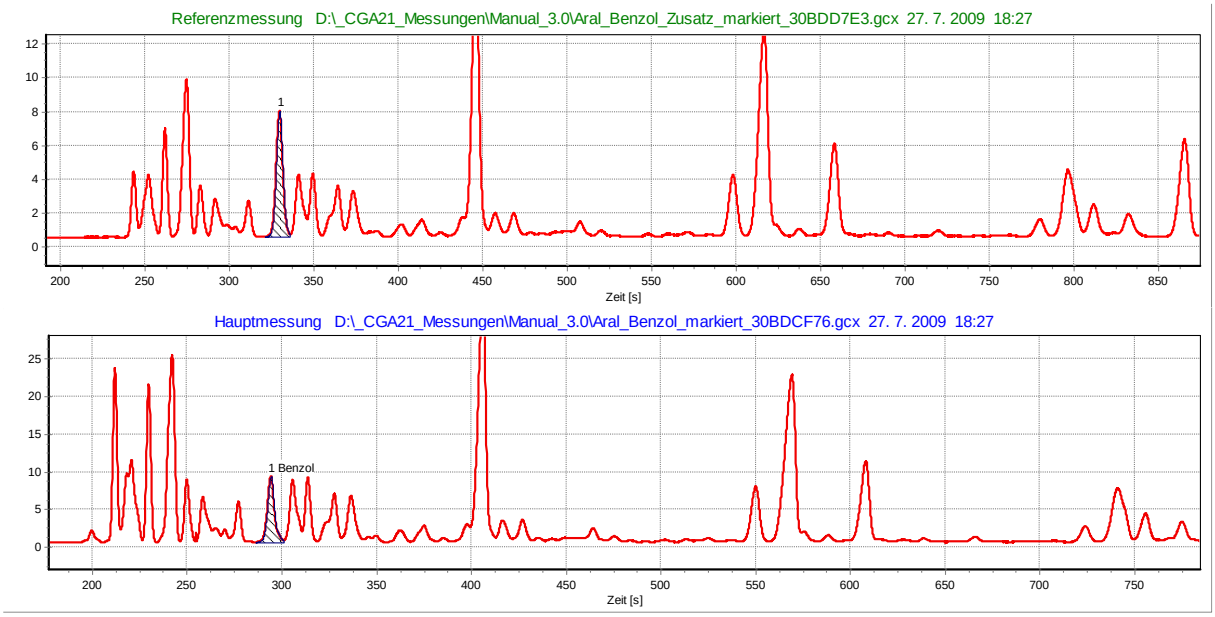

Fig. 13: Comparison of gasoline and gasoline with benzene added

For the sake of convenience, the peaks in Fig. 13 already have been marked. Fig. 14 shows an enlarged section of the region where the benzene is expected. If, however, we assume

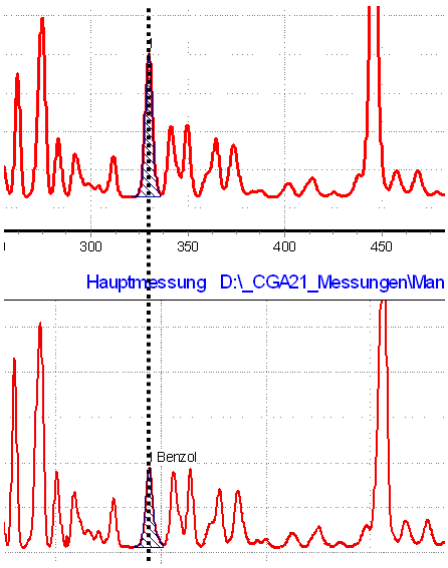

Fig. 14: Section from Fig. 13

there is a substance different from benzene with a retention time very close to that of benzene we would be misguided. Our assumption about the identity of peak 1 in the diagram only has a certain probability and is far away from certainty. To come closer to this, additional analysis would be necessary, e. g. a mass spectrum of the peak would give clear statement about the nature of the substance behind it.

Quantitative analysis:

Now that we know the peak that represents the benzene, the next step is the determination of the content of the sample. This can be done in two different ways:

- Comparison of total peak area and single peak area: Estimation of the content by comparing the area of all peaks with the area of the desired peak.
- Determine the absolute mass through a comparison with the peak of the reference substance

3.3.2 Comparison total area / single peak area

Quick and dirty method for a rough estimate. Step by step procedure:

- Mark the benzene peak.
- Mark entire chromatogram before the benzene peak and after the benzene peak. See Fig. 15. The benzene peak is marked for clarity.
- The peak list now shows three peaks. One of the peaks is benzene, the other peaks represent all hydrocarbons present in the probe. The percentage of benzene can now be read directly from the peak table. Although we obtain a rasonable value of 2,7%, it must be stressed that this can be regarded only as a rough estimate. The reasons are manifold. One of the most important is the fact, that the sensitivity of the flame ionisation detector is dependent on the nature of the analysed substances.

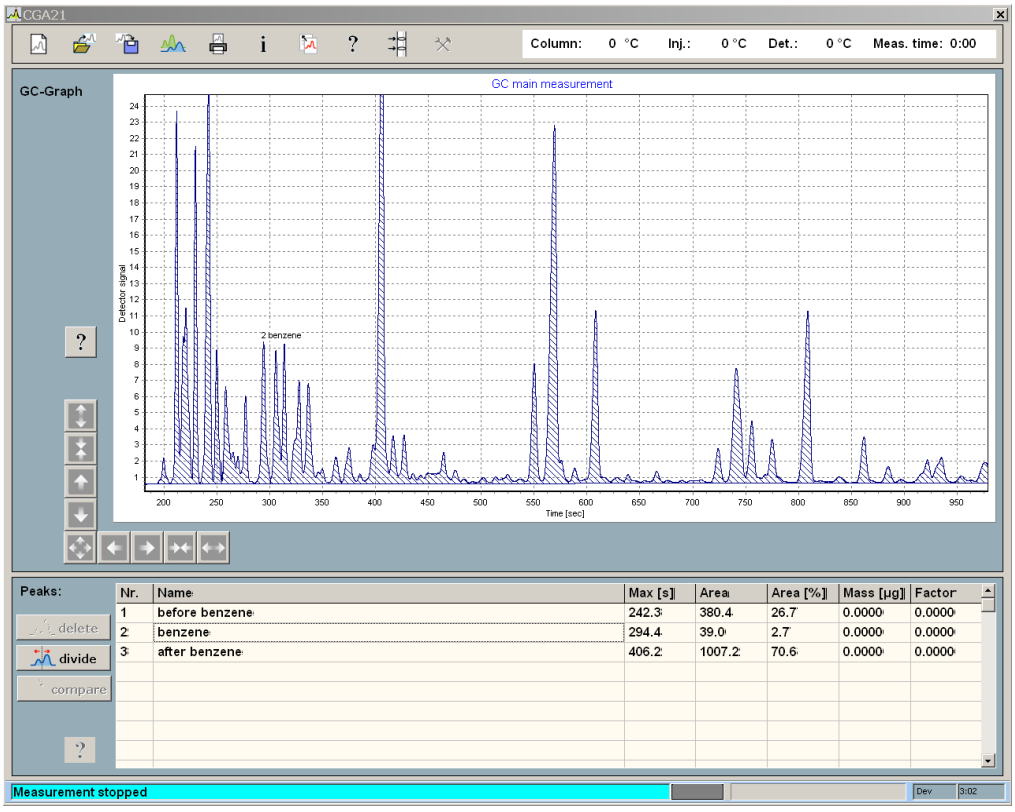

Fig. 15: Comparison total/single peak area

3.3.3 Determine the absolute mass through comparison with a reference substance

The classical procedure to determine the quantity of a substance in a sample mixture is to make two measurements and compare the peak areas of interest:

1. Perform a measurement using the substance containing the benzene in an unknown concentration, termed ***main measurement*** *here****.*** The benzene peak must be identified as shown in Chapter 3.3.2. Mark the benzene peak.
2. Perform a measurement using a standard solution containing benzene in a known concentration. This measurement is termed ***reference measurement*** here. Mark the benzene peak. It is recommendable to use a concentration roughly equal to the expected value, i.e. 4% in this case. For comprehension use file *Heptane_benzene_mark_quant.gcx* **The two measurements should be performed under identical conditions** (T-program, gas flow etc). For best results you should make two consecutive measurements.
3. Assign the mass of the benzene contained in the injected reference substance to the benzene peak in the reference measurement.
4. Compare the areas of the reference benzene peak with the identified benzene peak in the main measuremet.

For clarity steps 3 and 4 from above are explained in full detail:

Step 3:

- Click “open measurement” and load the **reference** measurement into the main display.
- Mark the benzene peak by double clicking start and end position of peak. The peak now appears as a line in the peak list.
- Double click the name field and enter “benzene”, then double click the mass field and enter 17.5 The mass of the injected portion of benzene is calculated from the volume of the injected mixture, its content, and the density of the benzene:

  $$m(\text{Benzene}) = V(\text{Injection}) \cdot w(\text{Benzene}) \cdot \rho(\text{Benzene})$$
  $$m(\text{Benzene}) = 0.5\ \mu l \cdot 4\% \cdot 0.874\ g/ml \approx \underline{17.5\ \mu g}$$

- The benzene line in the peak table is now updated and the color of the benzene peak turns to green, meaning that this peak is calibrated with a known mass.

The chromatogram of the reference measurement is shown in Fig. 16. The benzene has already been marked and assigned peak mass and name.

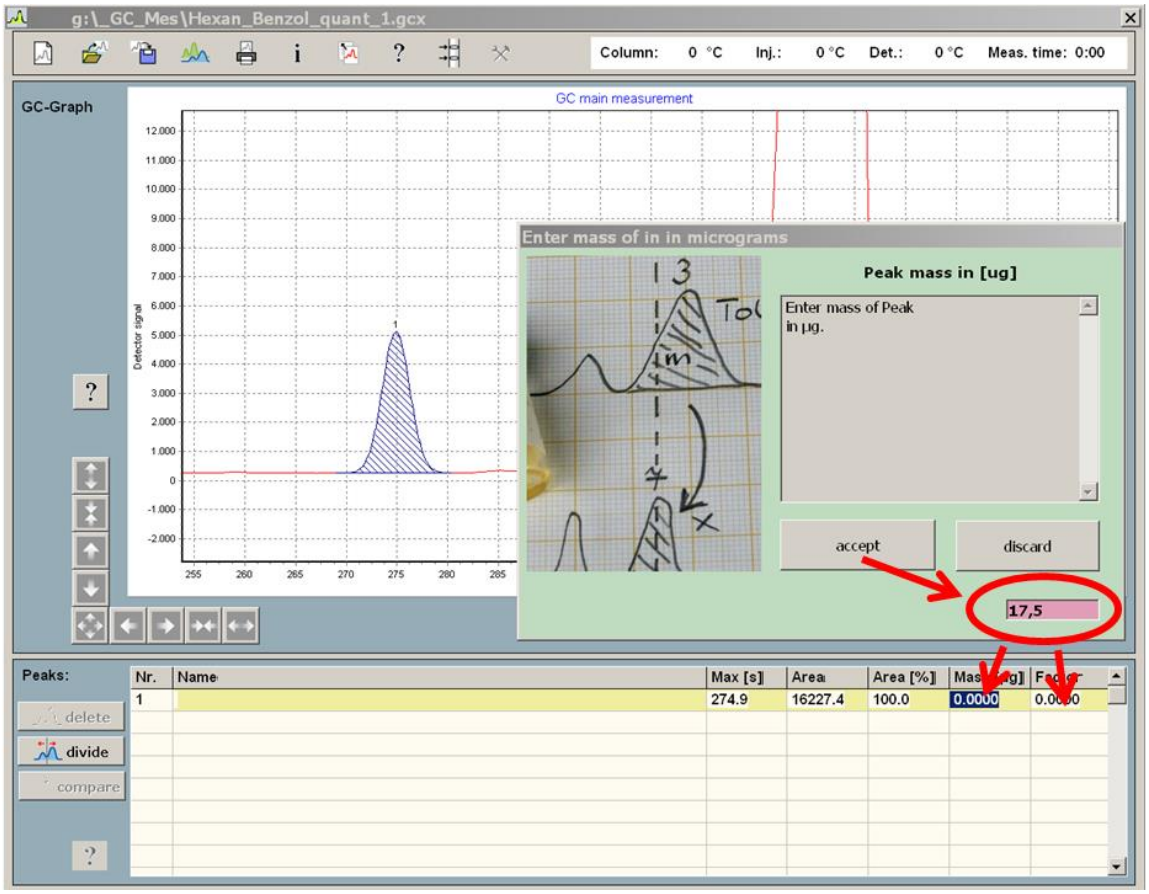

Fig. 16: Refererence measurement 'benzene in heptane', entering peak mass

Step 4:

Now we can finally compare the gasoline measurement and the benzene reference measurement:

- Open the **main measurement** by clicking "open measurement" in the tool bar on top
- Click "compare measurement" in the tool bar, now two data displays are visible.
- Open the reference measurement by clicking the "open reference" button on the left hand side of the reference display.
- Adjust the display excerpt to suit your requirements. The two selected peaks should now be roughly at the same position, as shown in Fig. 18. If this is not the case, use the pan and zoom buttons to move both maxima into about the same position.
- Click the "compare" button left to the peak list, move the cursor into the main measurement display where it turns into a line cursor. If the line cursor is inside the benzene peak, click the left mouse button.
- This triggers the following actions: From the known mass of the reference peak and its area a factor is determined that allows for conversion of peak area to absolute mass. This factor is immediately used to compute the mass of the benzene peak in the gasoline probe. You should give this peak a name by double clicking the name cell and entering the name in the pop up window. Now don't forget to save your measurement!

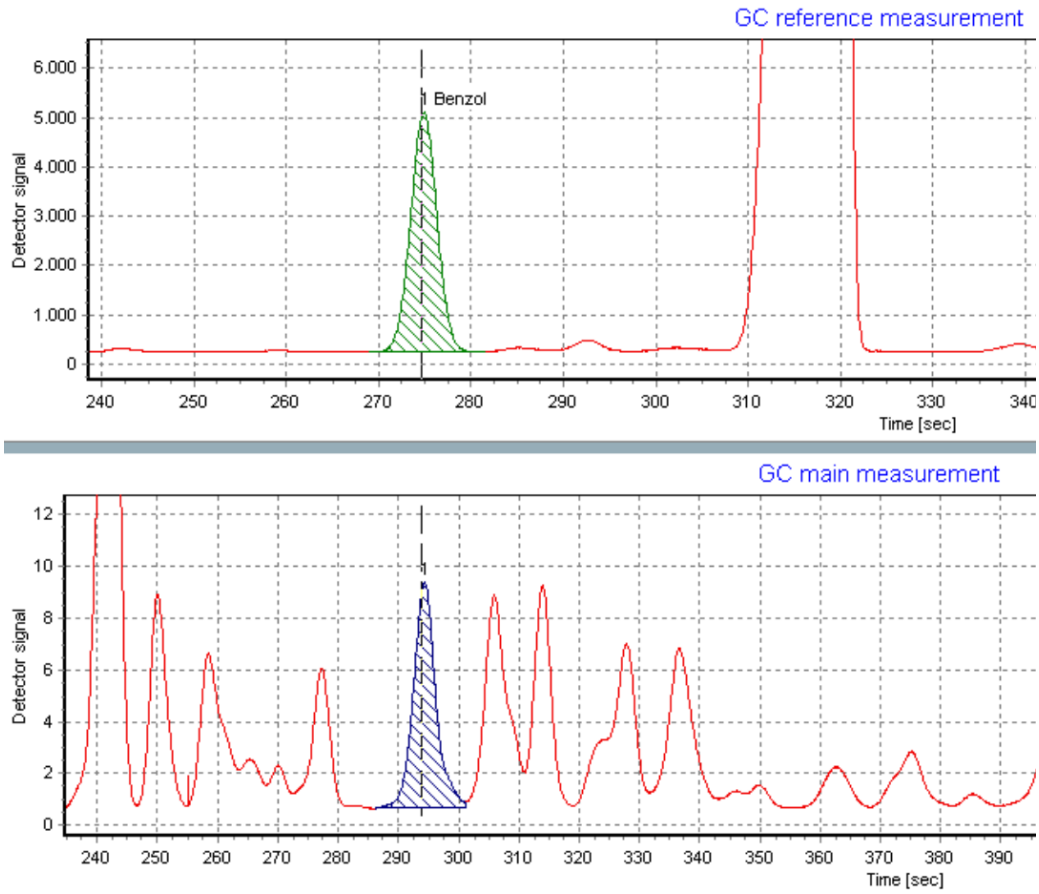

Fig. 17: Comparing refererence measurement and main measurement

You have determined the mass of benzene present in our sample of supergrade petrol. By balancing a simple equation, you can now calculate the volume percentage of benzene in the supergrade petrol:

m(Benzene) = 8.342µg

v(Benzene) = 8.342µg / 0.874g/ml = 9.54nl

w(Benzene) = 9.54nl/0.5µl ≈ 2.0 Vol%

Chapter 4 - Basic principles of gas chromatography

Chromatography in the gas phase is one of the cornerstones of instrumental analysis. After rapid development in the Seventies and Eighties of the last century, this technique has now, with the help of digital electronic and data processing, established itself on a highly advanced level. Suitable for all mixtures which can be vaporised without resulting in chemical changes, it even allows the separation of complex mixtures of substances in very small portions. In many cases, the retention times can be used to identify individual components; the peak areas allow quantitative determination after calibration.

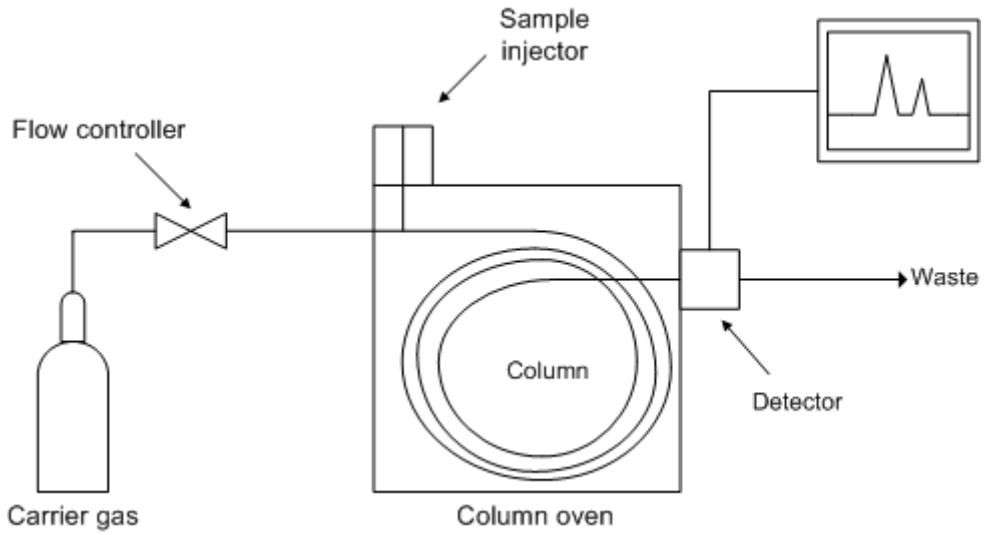

Fig. 18: The operating principle of a gas chromatograph (Wikipedia)

4.1 Principle of operation

An inert carrier gas, usually hydrogen or helium, flows in succession through a pressure control unit , injector, separation column and detector . The mixture to be separated is introduced into the heated sample injector with a microlitre syringe and vaporised there. The gaseous components of the mixture are transported by the carrier gas into the column and subsequently into the detector. The column separates the gaseous components spatially by retaining them for differing periods. As a result, these components emerge from the column at different points in time. When a component of the mixture emerges from the column, the detector issues an electrical signal which is recorded as a function of time. Earlier, this was done using a laboratory recorder; nowadays, the signal is converted into digital form and stored on standard media (e.g. hard disks or flash memory).

4.2 The chromatographic separation principle

4.2.1. Distribution

The process of distribution is best elucidated by means of a small experiment. A few crystals of iodine are added to a potassium iodide solution having a concentration c = 1mol/l. When shaken, the iodine crystals dissolve, turning the solution brown. If the solution is overcoated with a layer of hexane, the iodine particles diffuse through the phase limit into the hexane, turning it violet. After that, the experiment is performed in reverse order. A small quantity of iodine is dissolved in hexane and undercoated with a potassium iodide solution. This time,

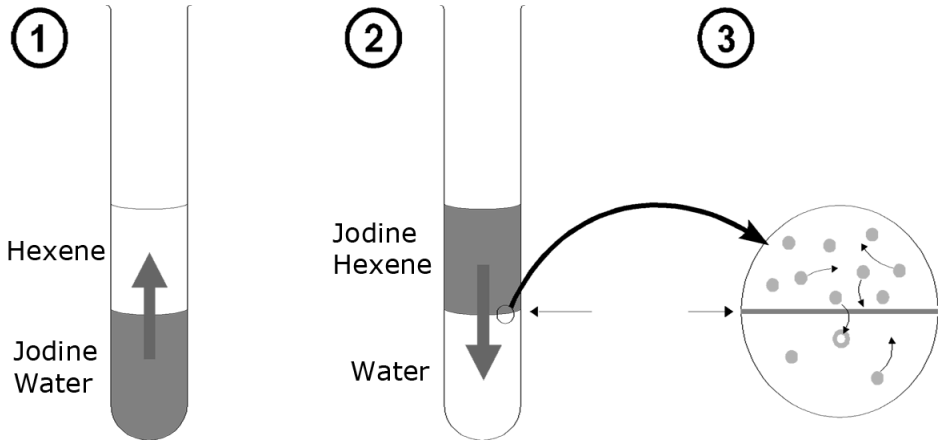

Fig. 19: Distribution between two phases

the iodine diffuses from the alkyl phase into the aqueous phase. Both these initial situations classically result in a dynamic equilibrium. If one waits long enough in both cases, the number of particles crossing the phase boundary from "top" to the "bottom" and vice versa turns out to be identical per unit of time, i.e. the concentration of particles remains unchanged over time in both phases.

According to NERNST, the following equation applies to small values of c in this case:

c(iodine in phase 1) / c(iodine in phase 2) = const

Consequently, the ratio of concentrations is a constant. This value is also termed distribution coefficient.

4.2.2 Distribution in the case of mobile phases

In an imaginary experiment, several test tubes of the type shown above are now arranged consecutively, but it is additionally assumed that one of the phases is laterally movable (mobile phase) while the other one remains fixed (stationary phase). In addition, the mobile phase is assumed to be slow in comparison with the diffusion rate.

This imaginary experiment provides several results:

- The particles in such a system drift with the mobile phase, but more slowly, because they are only moved by the phase when they are actually in it.
- The range over which the particles to be separated drift through the column increases during the course of the experiment.
- The speed of the particles relative to the mobile phase is directly dependent on the distribution coefficient, because statistically, a particle present more often in the stationary phase remains at rest longer. For this reason, its average speed is less than that of a particle which is present more often in the mobile phase. The relative speed of the particles is thus dependent on the substances involved with the result that it can be used for analytical separation.

The distribution coefficient is strongly dependent on the temperature. This can be easily understood in mechanochemical terms, because as the temperature increases, so does the drift rate of the particles and, consequently, their tendency to remain in the gaseous state (where the entropy is higher). This property is frequently made use of in chromatography.

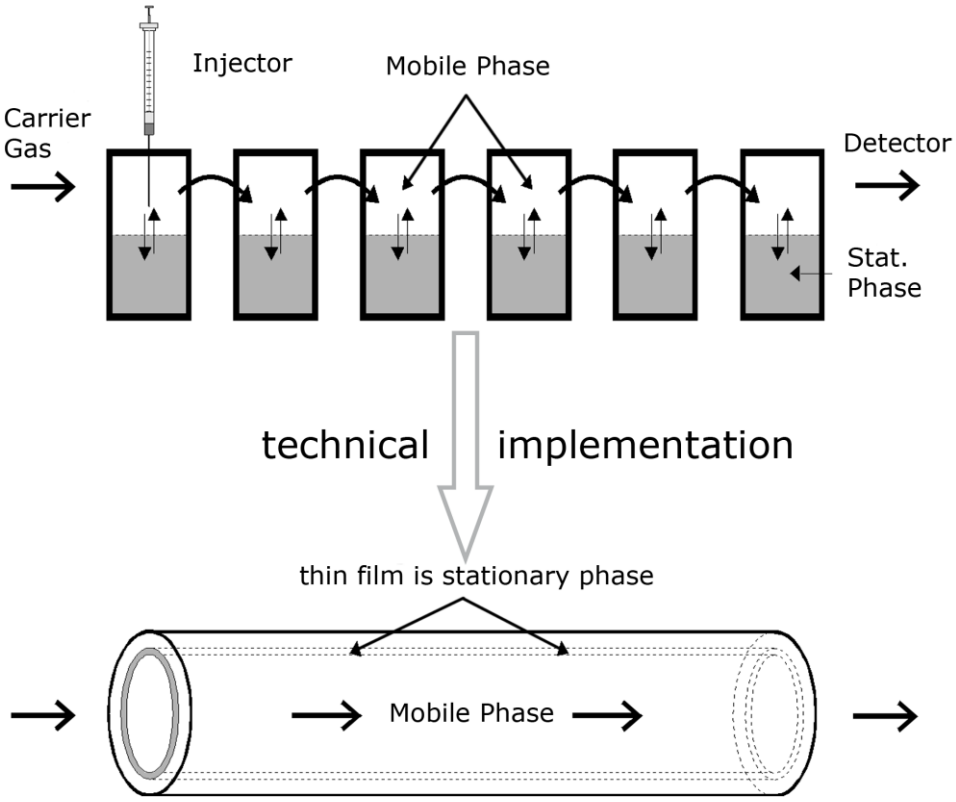

Fig. 20: Distribution in mobile phases

If the components in a mixture to be separated have widely differing boiling points, it might not be possible to separate all the individual substances given a constant column temperature. Either the substances with higher boiling points take a very long time to emerge from the column (elution) when the column has a low temperature, or the substances with lower boiling points are not separated when the column has a high temperature. This can be remedied by programming the temperature of the column furnace.

In practice, measurements are usually commenced at a column temperature matched with the most volatile component. After a phase of constant temperature (t0-t1), the column temperature is made to increase linearly over time (t1-t2) to allow elution of the less volatile components from the column. After this linear heating phase, the temperature is usually adjusted back to a constant level (t2-end). This type of temperature programming for the column furnace is a widely used technique nowadays.

Fig. 4 shows a standard temperature program. The heating rate $\Delta T/\Delta t$ is stated in degrees/minute; values here typically lie between 1 and 10 K/minute

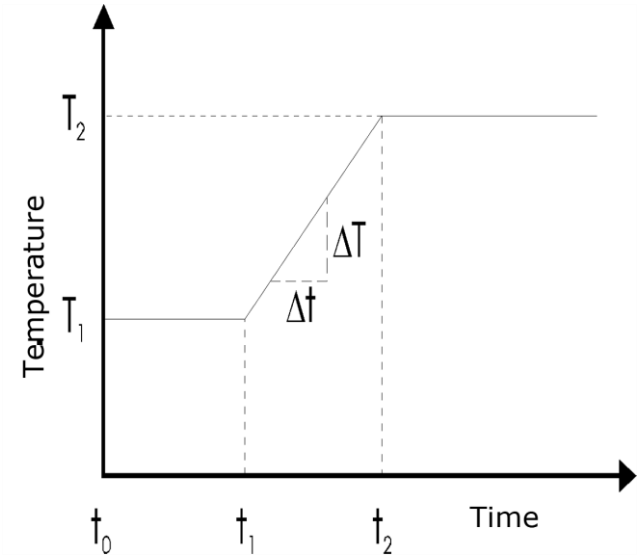

Fig. 21: Temperature program

Chapter 5 - Technique of gas chromatography

The following section describes the individual components of a gas chromatograph, their technical design, and provides notes on their practical operation.

5.1 The injector

> "If the column is described as the heart of chromatography, then sample introduction may, with some justification, be referred to as the Achilles heel"
>
> V. Pretorius and W.Bertsch, HRC & CC, 6/1983, 64

This quote illustrates the fact that a patented solution for sample introduction in gas chromatography is still not available. Technically, it is not easy to introduce a small amount of liquid having a volume of 0.1-1 microlitres precisely and reproducibly into a system at excess pressure. Consequently, there are a large number of designs and techniques of sample introduction; the two most familiar are described as follows.

5.1.1 Injector without split

This type of sample introduction is based on a simple principle. A microlitre syringe is used to puncture the septum, a rubber-like membrane; the needle is inserted fully and the plunger is pressed down to inject a measured amount of liquid into the vaporisation chamber of the injector, whose temperature should be several tens of degrees higher than the boiling point of the mixture. The vaporisation chamber usually consists of a glass tube, or liner, with an internal diameter of 2-5 millimetres. An obstruction is usually positioned in the middle of the liner to intercept the drop of liquid ejected from the syringe. On this obstruction, the drop must then be vaporised fully and rapidly, if possible, into a small gas cloud. This procedure is also termed flash vaporisation. The gas cloud is then transported by a flow of carrier gas into the column, where the actual separation takes place.

This type of sample introduction has the following basic disadvantages:

- The drop of liquid is not vaporised instantaneously but over a certain time period. During this period, the flow of carrier gas disperses the gas cloud.
- The expulsion of the gas cloud from the injector into the column is a process comparable with a dilution series. The concentration profile of an injected sample at the outlet of the injector exhibits the typical, exponential characteristic of such processes over the last part. In principle, this applies to all sample introduction systems but is particularly evident in the case of this simple construction.

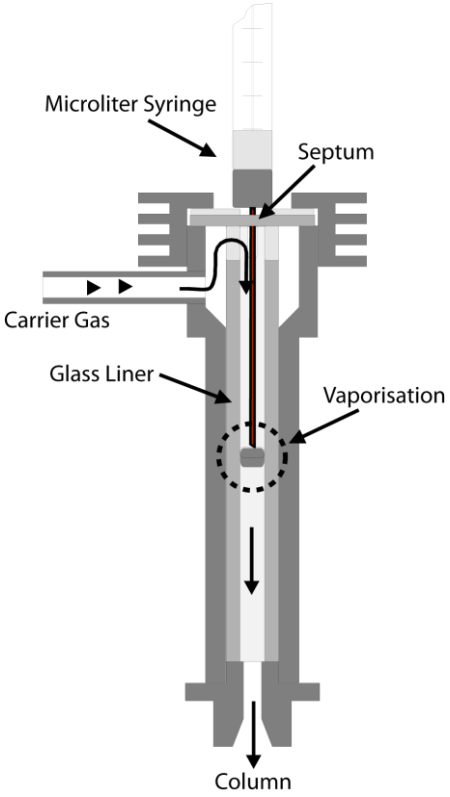

Fig. 22: Injector without split

5.1.2 Injector with split

This design allows the flow of carrier gas to be split after introduction of the sample. In this case, the flow of gas through the injector is much larger than through the separation column. Fig. 22 is meant to demonstrate this. First, the injected sample is vaporised. The gas cloud is transported by the carrier gas to the split, where the gas flow is separated. The smaller

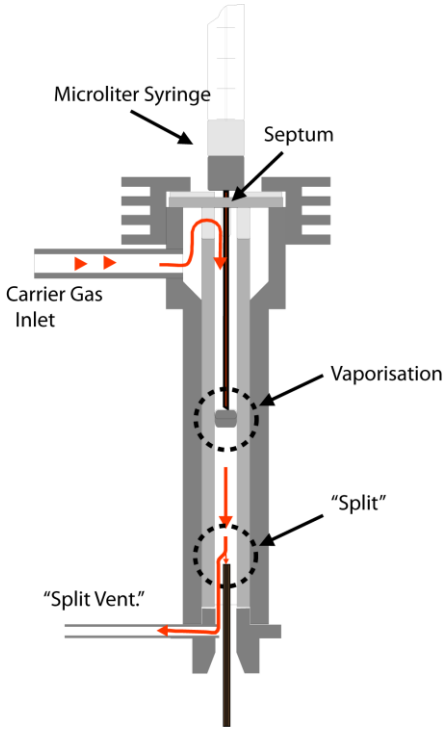

Fig. 23: Injector with split

portion flows into the column (usually consisting of a capillary column), while the larger portion escapes through the line leading to the split valve. The ratio of the gas flow through the column and the escaping gas flow is termed the split ratio. This value typically lies between 1:10 and 1:100. Two results are achieved by this process:

Due to the relatively high speed at which the gas cloud flows past the split point in the injector, the concentration-time characteristic of the test substances is much narrower, i.e. the tailing at the peak end is greatly reduced by the expulsion process. The absolute quantity of the test substance reaching the column is reduced by the same factor as the split ratio. This is a necessity for capillary columns, as their capacity for carrying substances requiring separation is several degrees of magnitude lower than that of packed columns. The following

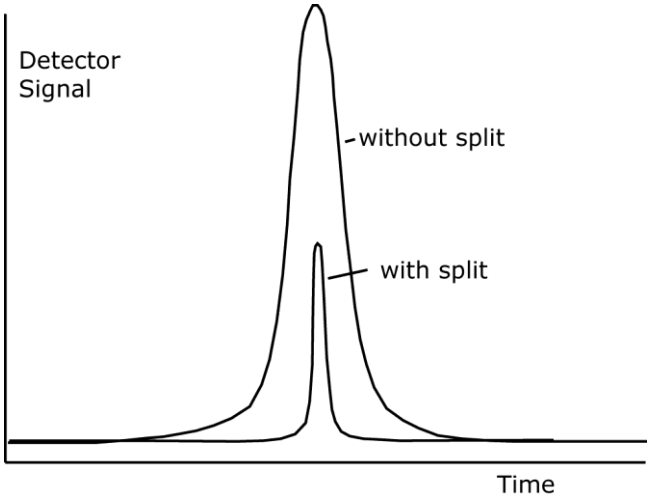

Fig. 24: Concentration profile with and without split

sample calculation is meant to elucidate this: Assume that a carrier gas flows through the column at a rate of 10 ml/min. and that the flow rate through the split outlet is 90 ml/min. When the gas cloud flows past the split point at a rate of 100 ml/min., only 10% of the injected sample will reach the column.

5.2 The separation column

5.2.1 Packed columns

This type of column consists of a chromium-steel or glass tube containing a porous material with a particle diameter of 0.05 to 0.5 mm which acts as the carrier and is coated with the stationary, liquid phase. The large surface area of this substance allows rapid attainment of the diffusion equilibrium between the mobile gaseous phase and the stationary liquid phase. Depending on the application involved, the proportion by mass of the liquid phases lies between 0.5 and 25%. Packed columns contain much more liquid phase than capillary columns and are thus very suitable for separating highly volatile components as well as large sample quantities,

particularly in conjunction with a thermal conductivity detector (TCD). Due to its design, the TCD is less sensitive than other detectors, such as the flame ionisation detector (FID). Standard version 1 of the device used here comprises a chromium-steel packed column with an internal diameter of 2 mm and a length of 2 m. Its carrier material consists of a preparation of diatomite with a grain size of 80-100 mesh and a coating of 5 % polydimethyl-siloxane (OV-1).

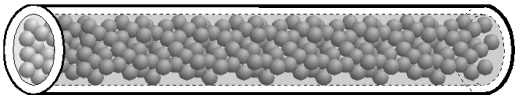

Fig. 25: Design of a packed column

5.2.2 Capillary columns

Nowadays, these columns usually consist of a thin-walled tube made of pure quartz SiO2, or fused silica. Columns like this are also termed open tubular columns (OTC). Here, the stationary phase consists of a thin film applied to the inside of the tube wall. Often, this film is also fixed to the silicon dioxide by means of chemical bonds. This allows the columns to be washed using suitable solvents without loosening the lining. For mechanical protection, the columns are usually equipped with a plastic coating of polyimide. Capillary columns are commercially available in graduated internal and external diameters:

Internal ∅ •	External ∅
0.10mm	0.27mm
0.20mm	0.35mm
0.25mm	0.38mm
0.32mm	0.48mm
0.53mm	0.75mm

The film thickness of the stationary phase varies between 0.1 and 5 micrometers. The columns have a standard length of between 10 and 50 meters. The above-mentioned distribution characteristics only apply approximately to low concentrations. If a column is overloaded in an attempt to separate large quantities of substances, distorted peaks occur, thus impairing the separation efficiency. It is clearly evident that columns with a thicker stationary phase can handle larger quantities of substances for the purpose of analysis. On the other hand, the diffusion processes forming the basis of separation are generally faster and more extensive if small dimensions are involved, i.e. thin columns with small film thicknesses for the stationary phase provide higher separation efficiency.

A compromise needs to be struck between these two conflicting requirements. This compromise is closely related to the detector used, as the substances emerging from the column need to generate an electrical signal in the detector. In any measurement circuit however, a detector always has the fundamental property of generating an interference signal which is termed noise. Just like weak signals from broadcasting stations easily fade on the radio, a very small peak is easily drowned by the inherent noise in the detector circuit. Put differently: Sensitive detectors like the flame ionisation detector allow the use of low-capacity columns which have a high separation efficiency, whereas a thermal conductivity detector, for example, requires the use of high-capacity columns due to its much lower sensitivity. Version 2 of this device, for example, uses a micro TCD in conjunction with a capillary column with an internal diameter of 0.53 mm and a film thickness of 5 micrometers.

5.3 Thermal conductivity detector (TCD)

The detector of a chromatograph is meant to output an electronic signal when a component of the substance to be analysed emerges from the column. There are diverse methods of generating such electrical signals, one of them being the thermal conductivity detector described in the following.

The ability of gases to conduct heat depends directly on the mass of their molecules. According to the kinetic theory of gases, light molecules are better conductors of heat than heavy ones. This fact is made use of by the TCD. Fig. 27 shows its fundamental design. The carrier gas from the separation column flows through a cylindrical chamber containing a thin wire made of a tungsten alloy. This wire, or filament, has an electrical resistance R which depends on the temperature of the filament. In the case of metals, this resistance generally increases with the temperature. Such conductors are said to have a positive temperature coefficient (PTC). If a current I flows through the resistance, the voltage V_{Det} through it is determined using Ohm's law:

$$V_{Det} = R(T) \bullet I$$

If a component with a higher molecular mass than that of the carrier gas emerges from the

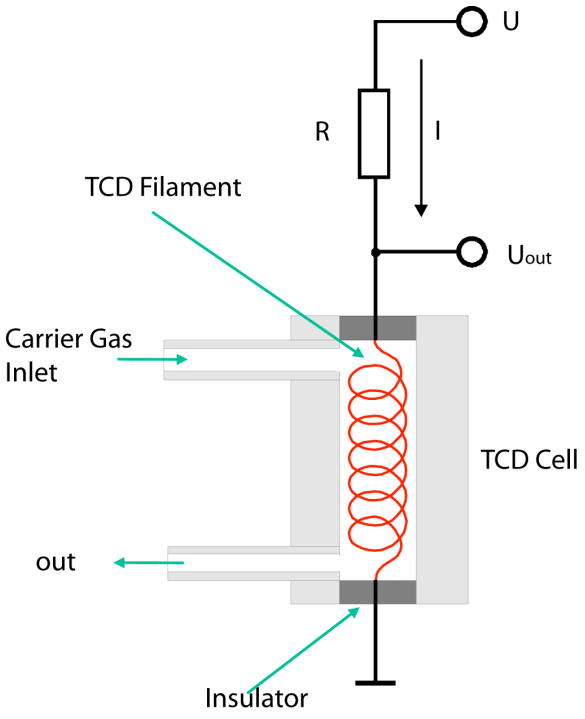

Fig. 26: Principle of the TCD

column, the heat from the heating filament is channelled off more slowly by the gas mixture. Consequently, the temperature T of the filament rises, and so does its electrical resistance. Given a constant current I, U_{Det} also rises as a result. This configuration hence indicates changes in the composition of a carrier gas in the form of voltage variations.

However, one disadvantage of this simple detector is that any other extraneous factor influencing the temperature of the filament also influences the voltage UDet and is thus superimposed on the effective signal. Examples of such undesirable effects are:

- Change in the flow of gas
- Variations in the operating voltage
- Change in the ambient temperature

In practice therefore, the detector is not used in the form described above. The configuration shown in Fig. 28 proves much more practical. In this case, the carrier gas flows through a

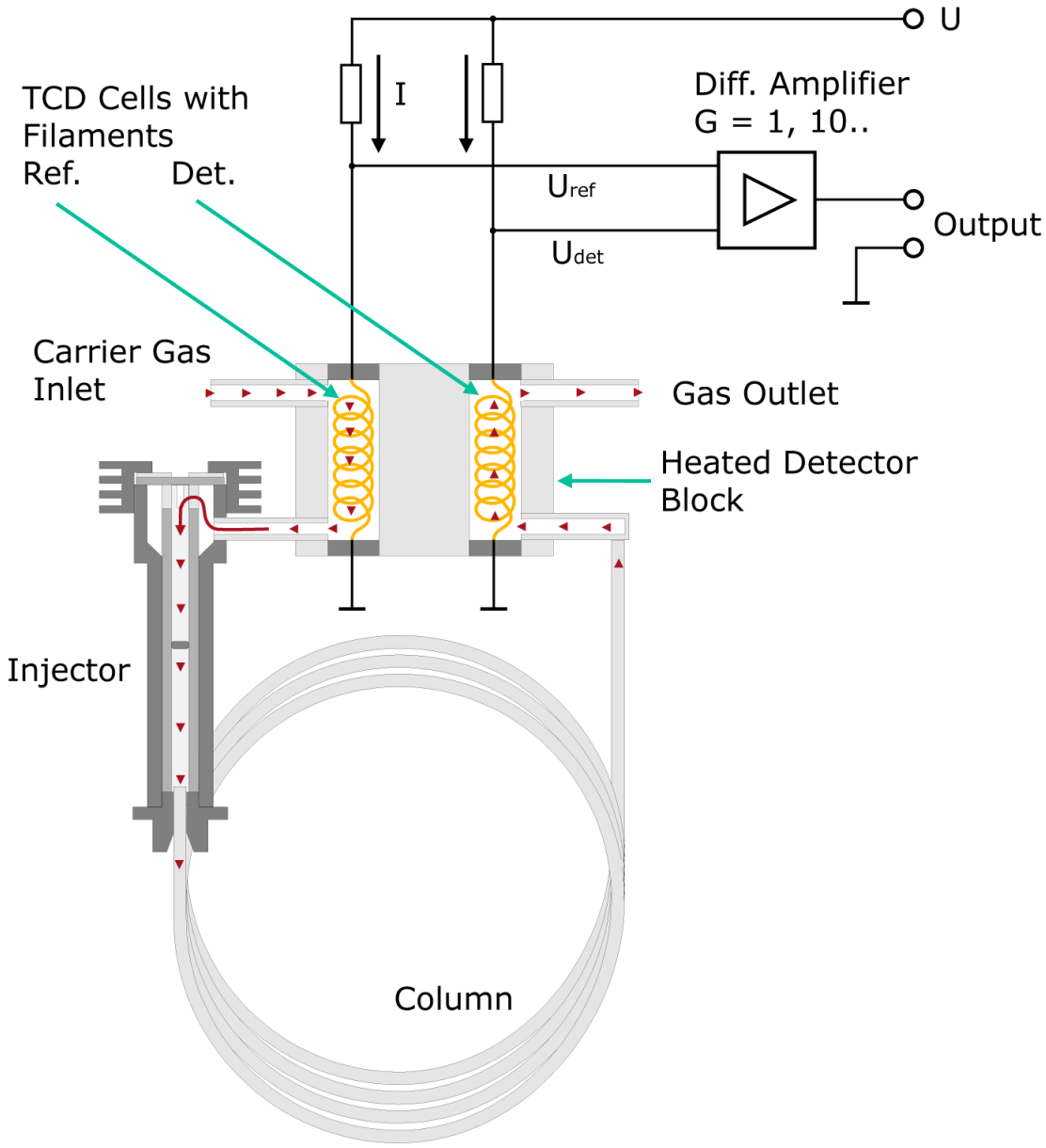

Fig. 27: Realisation of TCD with reference cell

second measurement cell before entering the injector. Both measurement cells are located together in a metal block possessing a high thermal conductivity. Here too, the current I results in a voltage drop U_{Ref} across the heating filament. In the absence of a test substance, U_{Det} should be equal to U_{Ref} if both the tungsten wires are identical and thus have the same resistance. The bridge voltage U_{Det}-U_{Ref} should be zero. This no longer applies to the emergence of a component from the column. Now a difference arises between U_{Det} and U_{Ref}. In a first approximation, this difference is proportional to the concentration of the expelled component. The voltage is amplified and recorded as a function of time.

Why is this detector much less sensitive to changes in the variables mentioned above? For example, let us assume that the ambient temperature rises. As the filaments are thermally coupled, this rise affects both of them in equal measure. Consequently, U_{Det} and U_{Ref} rise, the difference between these two values ideally remaining constant. Ideally speaking, changes in the flow of the carrier gas do not affect the bridge voltage either, as such changes influence

the temperature of both filaments, i.e. U_{Det} and U_{Ref} change accordingly but the difference between them remains nearly constant.

Summary of the essential properties of a TCD:

- At low concentrations of foreign substances, the output signal is proportional to the concentration.
- The sensitivity of a TCD depends on the substances involved.
- The dimensions of the measurement cell of a TCD must remain above a certain limit. The minimum diameter of such cells is approx. 2 mm; the volume is relatively large for gas flows in standard capillary columns. A TCD can only be used successfully in conjunction with capillary columns if these columns have a sufficient width (e.g. 0.53 mm internal diameter) and high film thickness (e.g. 5 micrometers).
- TCDs are non-destructive, i.e. the eluted substances are not changed.
- TCDs are not particularly suitable for trace analysis.
- TCDs should only be used with carrier gases possessing a high thermal conductivity. Hydrogen and helium are suitable for this purpose.
- If the temperature of the column furnace has been programmed, the reference line can be expected to drift.
- In the hot state, the tungsten filaments of the TCD might be adversely affected through oxidation by oxygen in the carrier gas. This can happen, for example, if the flow of carrier gas has inadvertently not been turned on. On no account must the TCD be operated with air for extended periods!

5.4 Flame ionisation detector (FID)

The flame ionisation detector used in your device is much more efficient than the thermal conductivity detector described previously. Its operation is described in this chapter.

If a hydrogen flame is placed between the plates of a charged capacitor as shown below, a low electrical current of a few hundred femtoamperes (10^{-15}A) flows through the circuit. It is assumed that thermal ionisation gives rise to free electrons and ions, although the yield of

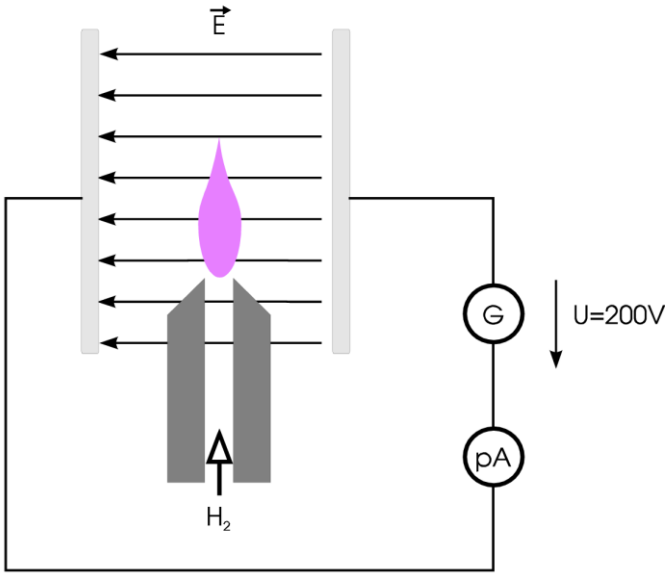

Fig.28: Operating principle of the FID

charged particles is very low. However, if the flow of hydrogen in such a configuration includes substances containing C-H bonds, e.g. alkanes, the current rises sharply. This is attributed to the formation of CHO+ ions and free electrons from CH• radicals. However, the yield of ions is very low in this case too. On average, only a handful of charge carriers are obtained from 500 000 atoms of carbon; nevertheless, this effect can be used successfully to detect hydrocarbons and related compounds. The schematic diagram below shows the technical design of the FID. The gas mixture to be analysed emerges from the capillary column right below a metallic nozzle (1). From there, it is transported by the hydrogen flow to the flame. Purified air is fed to this flame via separate channels. A circular electrode (2) is located above the flame; this electrode together with the nozzle, the voltage source (3) and the current-voltage converter (4) forms a closed electrical circuit. This detector must be heated to prevent condensation of the substances eluted from the column and of the H_20 produced in this process.

Summary of the essential properties of an FID:

- The most widely used type of detector with a simple, robust design
- Only detects substances containing C-H bonds
- Requires hydrogen and purified air (free of hydrocarbons) for operation
- Needs to be ignited

- High sensitivity and measurement response (ratio of the smallest to the largest effective signal)
- Sensitivity depends on the type of the substances detected
- Relatively insensitive to fluctuations in temperature and gas flow

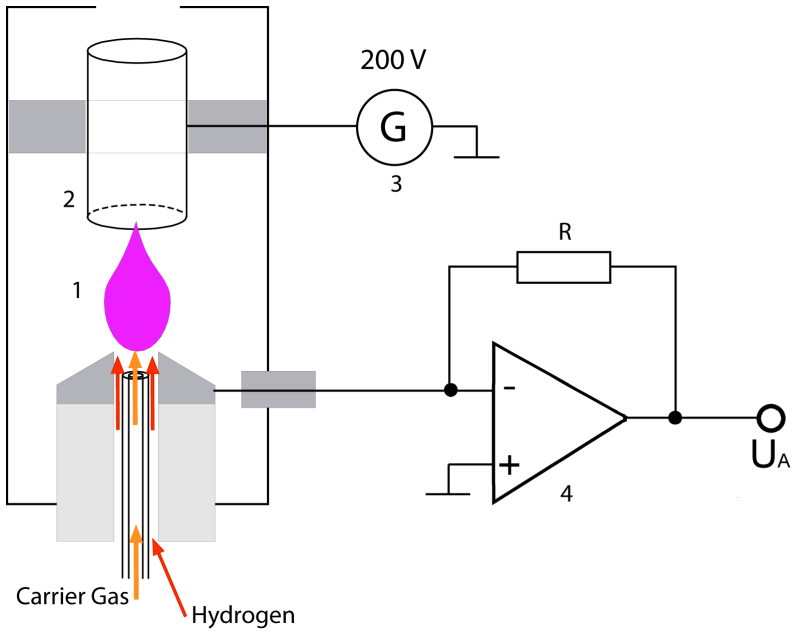

Fig.29: Technical design of the FID

5.5 Carrier gas flow

5.5.1 Type and purity of the carrier gas

In general, the following types of gas can be used for the operation of capillary columns: hydrogen, noble gases, e.g. helium, and nitrogen, whereas the higher the molar mass, the more suitable the gas. This is because gases with a high molar mass have a low diffusion rate, thus increasing the separation efficiency of a column in theory. In practice, this difference is only noticeable at very low flow rates and column admission pressures. The advantage of hydrogen as the carrier gas is that only one gas needs to be used in this case. Due to the low viscosity of hydrogen though, even small leaks into the system lead to considerable losses of hydrogen gas. The screw connection between the column and injector is of particular importance here. The conical seals made of Vespel® tend to shrink somewhat during the first few heating cycles. Consequently, the column connections should always be tightened after the first two heating cycles. These sealing joints should be checked on a regular basis.

In general: The carrier gas should be as pure as possible; a purity grade of 5.0 (99.999%) is recommended here. The price difference compared with lower purity grades is insignificant, but noticeable compared with purity grades higher than 5.0.

Oxygen is the enemy of all capillary columns. Particularly polar phases like Carbowax® are attacked even by small quantities of oxygen during heating. This leads to a continual worsening of the separation efficiency. If polar phases are used frequently, it is advisable to integrate an oxygen absorber in the carrier gas supply line. Such filters, e.g. the product Oxisorb®, are available commercially and stated in the list of accessories in the appendix.

5.5.2 Pressure control

This device does not have an integrated pressure control unit. If the pressure pump is equipped with a two-stage pressure reducer and the manometer on the low-pressure side has a sufficiently small measurement range (0-1.5 bar), a separate control unit is not necessary. The gas lines should not be made of rubber or PVC tubes, as these materials do not prevent the diffusion of oxygen to a sufficient degree. Teflon or copper tubes with screw connections are suitable; adapters for this purpose are available as accessories.

5.5.3 Adjusting the column admission pressure

Fig. 30 shows the separation efficiency of a column as the function of the gas flow. This graph - a modified display of the Van Deemter function- shows that the gas flow in a separating process can be optimised. This optimal value must be determined empirically. From its peak point, the efficiency curve drops less sharply toward larger gas flows than toward smaller gas flows; therefore, when in doubt, select a flow rate (pressure) which is slightly higher than the optimal value. In the case of this device, the suitable admission pressure lies between 0.1 and 1.0 bar if helium is used. If

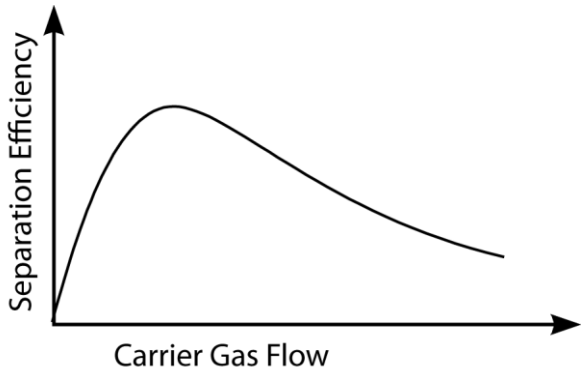

Fig.30: Separation efficiency vs. gas flow

hydrogen is used, a lower pressure must be selected, as this gas has a lower viscosity than helium.

Chapter 6 Maintenance work

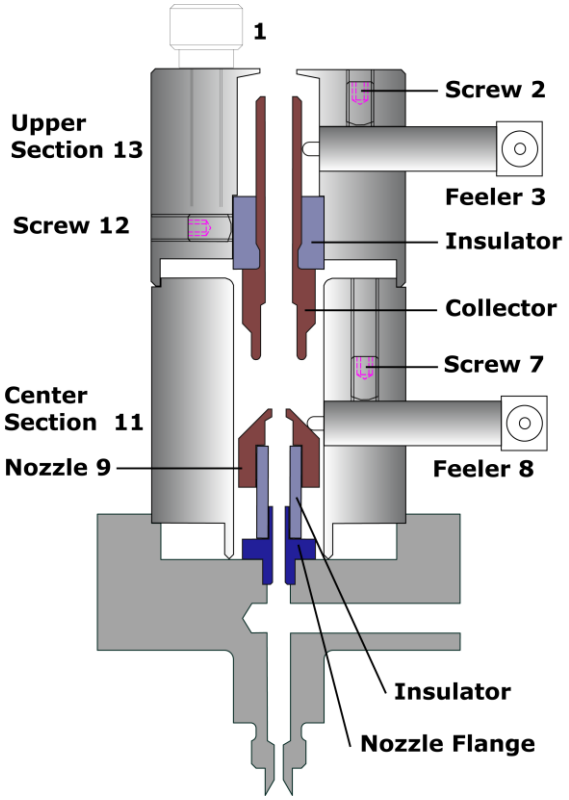

Fig. 31: FID Cross section

6.1 Disassembling the FID

There are two reasons for taking a FID apart:

1. Cleaning the FID from deposits after having used the GC for an extended period of time.
2. Measuring gas flows of carrier gas, hydrogen and air.

Necessary tools are included in delivery.

- Turn off power, pull mains plug and close gas valve at the pressure gas cylinder.
- Loosen socket set screw (2) and pull out the upper feeler (3). Loosen top screw (1) and take off upper section of the FID (13)
- Same way remove the lower feeler (8) by loosening screw (7).
- Turn out the two hexagon screws (not visible in Fig.) located on top of the center section (11), and take off center section.
- Now the nozzle (9) can be removed by **carefully** turning it **by hand.** The flange can be removed with an open end wrench size 8 mm.
- If necessary loosen the screw (12) sideways the upper section and pull out the isolator and the collector electrode.

Disassembling the FID step by step:

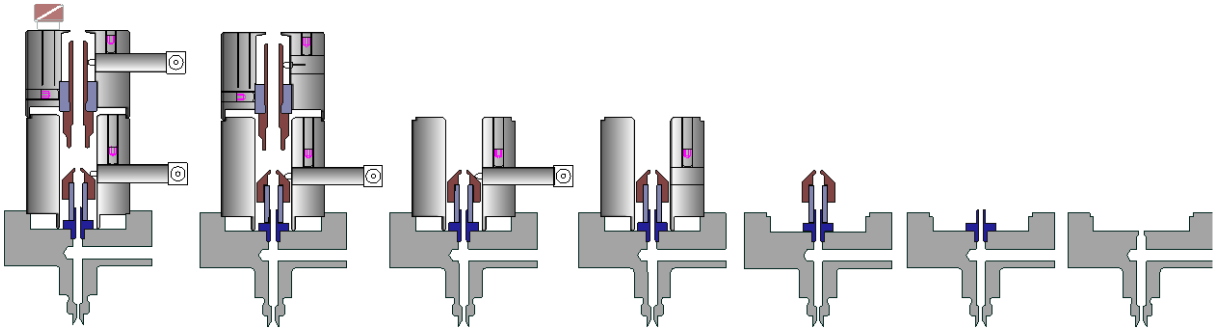

Fig. 32: Disassembling the FID

To assemble the FID repeat the steps above in reverse order. The connecting leads are marked: the cable with a red ring is to be connected to the upper feeler (3).

Important Note: Please take utmost care as to first mount the center section before pushing in and fastening the feeler (8). The same applies to the upper feeler (3). First mount the collector electrode (6) before inserting the feeler.

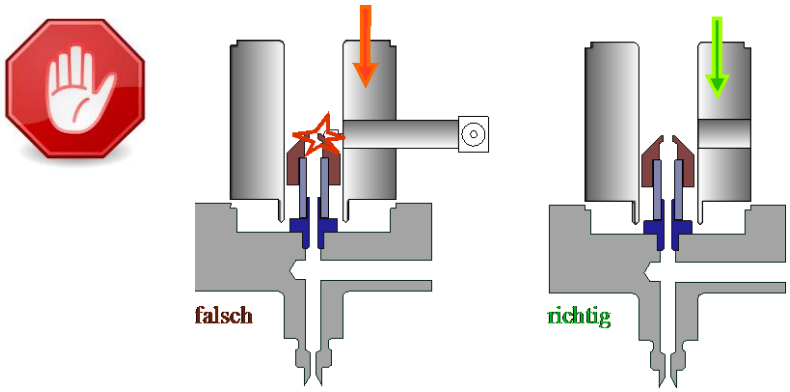

Abb. 33: Mounting the feelers

Cleaning the detector

All parts of the detector, except for the feelers, are very robust and can be cleaned mechanically with cleaning agents. **Do not wet the feelers!**

6.2 FID: Adjusting gas flows

Applies only for Chromatographs equipped with flame ionisation detector.

Factory settings

Nominal gas flows for FID:

Clean air gas flow: v(air) = 300 ml/min
Hydrogen gas flow: $v(H_2)$ = 30 ml/min

These values are factory preset for a hydrogene pressure of 0.3 bar (4.35 psi).

Working with a different FID hydrogen pressure from the above one requires readjustment of hydrogen gas flow. It is recommended to check for correct gas flows from time to time, in any case this has to be done if the FID does not work as expected, for example if ignition is not taking place or the hydrogen valve has been manipulated inadvertently.

Preparation

- Turn off GC. If the FID is still hot, wait until it can be touched by hand. Turn on the GC. After start up a message window appears. Select 'Measure gas flow' and the FID heating will stay turned off.
- Completely disassemble the FID as described in chapter 6.1. Remove the flange and the nozzle.

Measurement and adjustment of air flow

Screw the connector (included) into the right boring of the detector block. Slide the silicon hose that leads to the bubble flow meter over the connector tubing and measure the gas flow as described in chapter 6.8. Adjust the valve „Air" until the gas flow is between 280 and 320 ml/min. Usage of the bubble flow meter is explained in chapter 6.8.

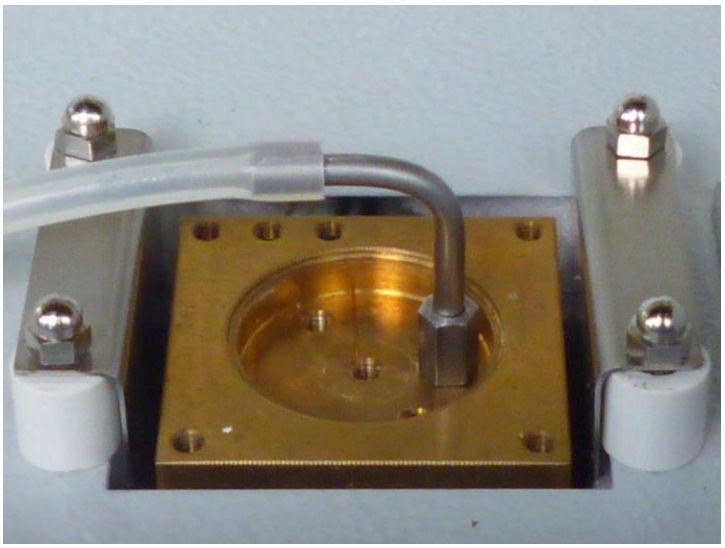

Fig. 34: Measuring air flow at the FID

Measurement and adjustment of hydrogen gas flow at the FID

- If the GC is operated with two different gases, eg. hydrogen and helium, close the helium supply.
- Set the hydrogen pressure to 0.3 bar (4.35 psi) at the pressure cylinder controls.

  - Screw the connector (included) into the center boring of the detector block. **Take care not to damage the end of the capillary column that protrudes from the FID block!**
  - Slide the silicon hose that leads to the bubble flow meter over the connector tubing and

- measure the gas flow as described in chapter 6.8. Adjust the air valve until the gas flow is between 28 and 32 ml/min

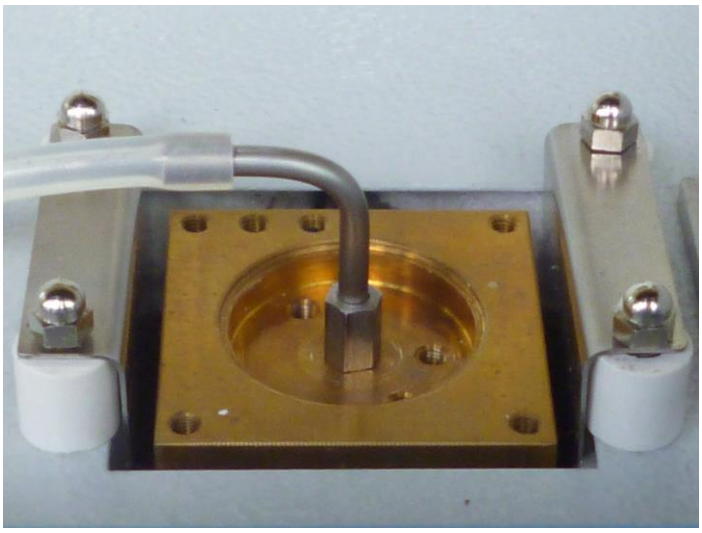

Fig. 35: Measuring hydrogen and carrier gas flow at the FID

Measurement of carrier gas flow (gas flow through the capillary column)

The carrier gas flow should be known to compute and adjust the split ratio. The measuring set-up is the same as in the previous section, but the procedure is different. **Advice: It is a whole lot simpler to compute the carrier gas flow or take the values from tables. See chapter 6.9**

- If the GC is operated with two different gases eg. hydrogen and helium, close the hydrogen supply.
- If the GC is operated with hydrogen only, close the hydrogen valve at the GC completely. **Don't forget to recalibrate the hydrogen gas flow after this measurement!**
- Set the helium pressure to 0.3 bar (4.35 psi) at the pressure cylinder controls.
- Screw the connector (included) into the center boring of the detector block. **Take care not to damage the end of the capillary column that protrudes from the FID block!**
- Slide the silicon hose, that leads to the bubble flow meter over the connector tubing and measure the gas flow as described in chapter 6.8. Note that for typical columns the gas flow is relatively small in the range of 0.2 – 2 ml/min.

6.3 TCD: Measuring and adjusting the carrier gas flow

Connect the exit of the carrier gas from the TCD (Thermal Conductive Detector) with the bubble flow meter by sliding the silicon hose that leads to the bubble flow meter over the connector tubing.

Adjust the pressure regulator at the gas cylinder to approx. 0.2 bar and wait 1-2 minutes to allow the carrier gas to displace the air in the system. Carrier gas flow should be between 10 and 50 ml/min. See chapter 5.5.3 for more information about carrier gas flow and separation efficiency. Usage of the bubble flow meter is explained in chapter 6.8.

6.4 FID: Adjusting the split ratio

General remarks

Capillary columns can only separate small amounts of substances. Using normal microliter syringes it is not practical to inject smaller amounts than approx. 0.5 µl. Such being the case the amount of substance is once again reduced inside the injector in an adjustable ratio termed 'split ratio'. See chapter 4 for details. The split ratio represents the ratio between the amount of carrier gas entering the column and the amount leaving the injector unused through the so-called split vent. Split ratios from 1:5 to 1:200 are used. The ratio implies a tradeoff between sensitivity and separation efficiency that depends also on the type of column. In our case values of around 1:20 to1:50 are a good compromise.

Example:

The following describes the adjustment of a split ratio of 1:50

- The carrier gas flow rate must be known at the given pressure. It can be measured or calculated. In our example we assume the carrier gas flow to be v(column) = 1.3 ml/min.
- We multiply this flow rate by the split ratio 1:50 to get the flow rate through the split vent: v(split) = 50 * 1,3 = 65 ml/min.
- The bubble flow meter is then connected to the split vent
- We now measure the gas flow several times while turning the split vent valve until the flow rate is near the desired value of 65 ml/min. **The absolute value of the split vent flow rate is not important. It's rather the constancy between measurements that matters.**

6.5 Maintaining the injector

Changing the liner

A liner is a glass tubing inside the injector. After an extended period of operation this part can be contaminated or plugged. It should then be cleaned or exchanged. Liners are expendable items. It is not a bad idea to keep one or two handy as spare parts.

- Turn off power, pull mains plug and close all gas valves at the pressure gas cylinders.
- Unscrew the heatsink from the top of the injector.
- Remove septum and pressure plate from the heat sink.
- Pull out the liner with a suitable tool e.g. a small screwdriver.
- Before mounting the liner its outer surface should be carefully cleaned.
- Insert the glass tube with its **indentations directing upwards** into the opening of the injector. The upper end of the liner should be positioned on the same level as the rim of the injector.
- Put the septum and then the pressure plate on top of the injector opening. (Do not change the order!) Screw the heat sink onto the injector and fasten hand tight.

6.6 Exchanging the septum

If the septum shows signs of wear it should be replaced. Follow the directions of the previous chapter, but, of course, do not remove the liner. Durability of septa depends on injection technique and varies. Use only low bleed septa. A couple of spare septa are included.

6.7 Exchanging the capillary column

The capillary column is mounted inside the column oven with two hexagonal nuts. The ends of the column are connected to the injector (left hand side) and detector (right hand side) with ferrules and column screws. (see Fig. 36)

Removal of column

- Loosen column screws at both ends with wrench 8mm and pull out the ends together with screws and ferrules. In most cases the ferrules fit tightly to the capillary.
- Loosen two nuts M3 at the column cage and take out the column including ferrules and nuts.
- Ferrules and column screws should be left in place if possible. If you want to remove it, firmly grab the capillary between two fingers and pull the screw.

Owing to circumstances it can make sense to cut an inch or two from the end of the column. Lay the column end on to a flat surface and scratch the capillary with a carbide or diamond cutter, then bend it symmetrically over both thumbs until it breaks. Slide the screw and the ferrule over the capillary, the conical end of the ferrule must point to the end of the column, see Fig. 36 for details.

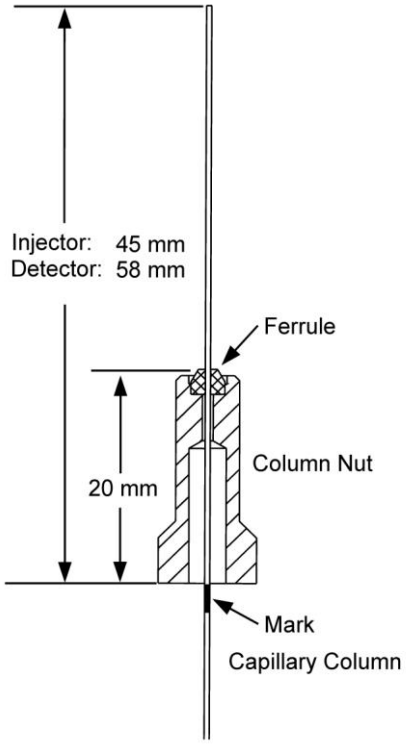

Fig. 36: Preparations when installing the capillary column

Connecting the column on the injector side

It is mandatory, to follow the measures of Fig. 36 above. To ensure this, it is recommended to mark the column with a felt-tip pen as shown. The column is inserted carefully into the injector. This procedure is delicate and requires some patience und manual skill.
Tighten the column screw by hand first, then fasten with wrench 7 mm. Pull slightly with two fingers at the column to check the column for firm seating. If doing so, the column must not move.

Important note: It is indispensable to retighten the column screws after one or two heating cycles! This is all the more important if hydrogen is in use as a carrier gas

Fig. 37: Connecting the column on the injector side

Connecting the column to the detector

The procedure is principally the same as described in the previous paragraph. If you encounter a barrier when inserting the column, we advise to move the column slightly back and forth to facilitate its way upwards until the mark is in its final position. **Regard warning above!**

6.8 Using the bubble flow meter

This simple but efficient flow meter essentially consists of a graduated glass tube in which a gas flow moves up a soap film.

Prior to first use, fill the rubber ball with water and add one drop of liquid kitchen detergent. Slide the rubber ball onto the lower end of the glass tube, wetting the glass end helps.

To initiate the measuring process, squeeze the rubber ball containing the detergent solution until the level of the liquid passes the gas inlet, which leads to the formation of one or more soap films.

Now select a measurement volume between two rings on the glass tube, start a timer when the film passes the first ring, stop the timer when it passes the second ring. Calculate the flow rate from the equation:

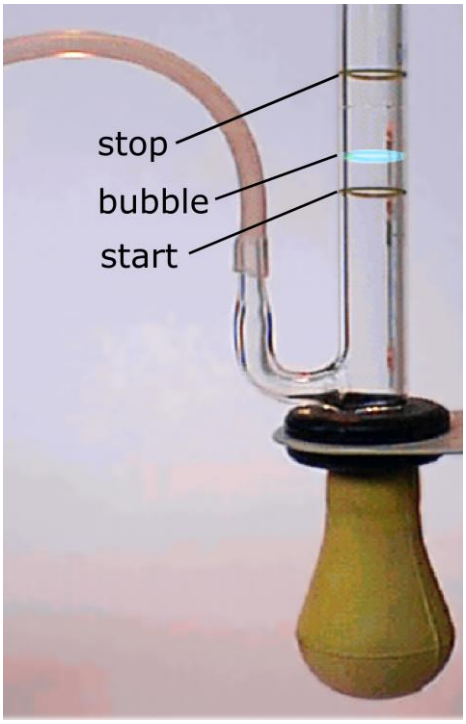

Fig. 38: Bubble flow meter

Flow rate: v = Volume/Time [ml/min]

Example: Elapsed time between two marks with a distance of 2 ml: t = 9,6 s.

v = 2 ml / 9,6 s = **0,208 ml/s**

v = 0,208 ml/s • 60 s/min **= 12 ml/min**

You can achieve this result much more easily by using the GC software. Simply click on “Measure gas flow” and use as directed.

6.9 Calculating the carrier gas flow

Capillary columns are thin tubes, for which Poiseuilles law applies. The law can be written in the form

$$dV/dt = v = (R/\eta) \cdot \Delta p \qquad (1)$$

where

$$R = k \cdot r^4/l \qquad (2)$$

"*Flow rate dV/dt of a gas through a thin tube is proportional to the pressure difference Δp"*

The factor R/η depends on the kind of gas, its viscosity, the radius r and the length l of the tube.

The following tables show calculated values for thin tubes of 0.32 mm diameter, 25 meters length at different temperatures for the following gas types: Hydrogen, helium, nitrogen. It can be used to estimate the carrier gas flow for capillary columns. Note that this is a first-order approximation.

Helium

Gas flow in ml/min through column 0,32 mm/25 m pressure p in bar, T in °C

p	0,1	0,2	0,3	0,4	0,5	0,6	0,7	0,8	0,9	1	1,1	1,2	1,3	1,4	1,5
T															
20	0,57	1,14	1,72	2,29	2,86	3,43	4,01	4,58	5,15	5,72	6,30	6,87	7,44	8,01	8,59
30	0,56	1,12	1,68	2,24	2,80	3,37	3,93	4,49	5,05	5,61	6,17	6,73	7,29	7,85	8,41
40	0,55	1,10	1,65	2,20	2,75	3,30	3,85	4,40	4,95	5,50	6,05	6,60	7,15	7,70	8,25
50	0,54	1,08	1,62	2,16	2,70	3,23	3,77	4,31	4,85	5,39	5,93	6,47	7,01	7,55	8,09
60	0,53	1,06	1,59	2,11	2,64	3,17	3,70	4,23	4,76	5,29	5,82	6,34	6,87	7,40	7,93
70	0,52	1,04	1,56	2,08	2,59	3,11	3,63	4,15	4,67	5,19	5,71	6,23	6,74	7,26	7,78
80	0,51	1,02	1,53	2,04	2,55	3,06	3,57	4,07	4,58	5,09	5,60	6,11	6,62	7,13	7,64
90	0,50	1,00	1,50	2,00	2,50	3,00	3,50	4,00	4,50	5,00	5,50	6,00	6,50	7,00	7,50
100	0,49	0,98	1,47	1,96	2,46	2,95	3,44	3,93	4,42	4,91	5,40	5,89	6,39	6,88	7,37
110	0,48	0,97	1,45	1,93	2,41	2,90	3,38	3,86	4,34	4,83	5,31	5,79	6,27	6,76	7,24
120	0,47	0,95	1,42	1,90	2,37	2,85	3,32	3,80	4,27	4,74	5,22	5,69	6,17	6,64	7,12
130	0,47	0,93	1,40	1,87	2,33	2,80	3,27	3,73	4,20	4,66	5,13	5,60	6,06	6,53	7,00
140	0,46	0,92	1,38	1,83	2,29	2,75	3,21	3,67	4,13	4,59	5,05	5,50	5,96	6,42	6,88
150	0,45	0,90	1,35	1,81	2,26	2,71	3,16	3,61	4,06	4,51	4,96	5,42	5,87	6,32	6,77
160	0,44	0,89	1,33	1,78	2,22	2,66	3,11	3,55	4,00	4,44	4,88	5,33	5,77	6,22	6,66
170	0,44	0,87	1,31	1,75	2,19	2,62	3,06	3,50	3,93	4,37	4,81	5,24	5,68	6,12	6,56
180	0,43	0,86	1,29	1,72	2,15	2,58	3,01	3,44	3,87	4,30	4,73	5,16	5,59	6,02	6,45
190	0,42	0,85	1,27	1,69	2,12	2,54	2,97	3,39	3,81	4,24	4,66	5,08	5,51	5,93	6,35
200	0,42	0,83	1,25	1,67	2,09	2,50	2,92	3,34	3,76	4,17	4,59	5,01	5,42	5,84	6,26
210	0,41	0,82	1,23	1,64	2,06	2,47	2,88	3,29	3,70	4,11	4,52	4,93	5,34	5,76	6,17
220	0,41	0,81	1,22	1,62	2,03	2,43	2,84	3,24	3,65	4,05	4,46	4,86	5,27	5,67	6,08
230	0,40	0,80	1,20	1,60	2,00	2,40	2,79	3,19	3,59	3,99	4,39	4,79	5,19	5,59	5,99
240	0,39	0,79	1,18	1,57	1,97	2,36	2,76	3,15	3,54	3,94	4,33	4,72	5,12	5,51	5,90
250	0,39	0,78	1,16	1,55	1,94	2,33	2,72	3,10	3,49	3,88	4,27	4,66	5,04	5,43	5,82

Hydrogen

Gas flow in ml/min through column 0,32 mm/25 m pressure p in bar, T in °C

p		0,1	0,2	0,3	0,4	0,5	0,6	0,7	0,8	0,9	1	1,1	1,2	1,3	1,4	1,5
T																
20		1,27	2,54	3,81	5,08	6,36	7,63	8,90	10,17	11,44	12,71	13,98	15,25	16,53	17,80	19,07
30		1,25	2,49	3,74	4,98	6,23	7,47	8,72	9,96	11,21	12,45	13,70	14,94	16,19	17,43	18,68
40		1,22	2,44	3,66	4,88	6,10	7,32	8,54	9,76	10,98	12,20	13,42	14,64	15,86	17,08	18,30
50		1,20	2,39	3,59	4,79	5,98	7,18	8,37	9,57	10,77	11,96	13,16	14,36	15,55	16,75	17,95
60		1,17	2,35	3,52	4,69	5,87	7,04	8,21	9,39	10,56	11,73	12,91	14,08	15,25	16,43	17,60
70		1,15	2,30	3,45	4,60	5,76	6,91	8,06	9,21	10,36	11,51	12,66	13,81	14,96	16,12	17,27
80		1,13	2,26	3,39	4,52	5,65	6,78	7,91	9,04	10,17	11,30	12,43	13,56	14,69	15,82	16,95
90		1,11	2,22	3,33	4,44	5,55	6,66	7,76	8,87	9,98	11,09	12,20	13,31	14,42	15,53	16,64
100		1,09	2,18	3,27	4,36	5,45	6,54	7,63	8,72	9,80	10,89	11,98	13,07	14,16	15,25	16,34
110		1,07	2,14	3,21	4,28	5,35	6,42	7,49	8,56	9,63	10,70	11,77	12,84	13,91	14,98	16,05
120		1,05	2,10	3,16	4,21	5,26	6,31	7,36	8,41	9,47	10,52	11,57	12,62	13,67	14,73	15,78
130		1,03	2,07	3,10	4,14	5,17	6,20	7,24	8,27	9,31	10,34	11,37	12,41	13,44	14,48	15,51
140		1,02	2,03	3,05	4,07	5,08	6,10	7,12	8,13	9,15	10,17	11,18	12,20	13,22	14,23	15,25
150		1,00	2,00	3,00	4,00	5,00	6,00	7,00	8,00	9,00	10,00	11,00	12,00	13,00	14,00	15,00
160		0,98	1,97	2,95	3,94	4,92	5,90	6,89	7,87	8,85	9,84	10,82	11,81	12,79	13,77	14,76
170		0,97	1,94	2,90	3,87	4,84	5,81	6,78	7,75	8,71	9,68	10,65	11,62	12,59	13,56	14,52
180		0,95	1,91	2,86	3,81	4,77	5,72	6,67	7,62	8,58	9,53	10,48	11,44	12,39	13,34	14,30
190		0,94	1,88	2,82	3,75	4,69	5,63	6,57	7,51	8,45	9,38	10,32	11,26	12,20	13,14	14,08
200		0,92	1,85	2,77	3,70	4,62	5,54	6,47	7,39	8,32	9,24	10,17	11,09	12,01	12,94	13,86
210		0,91	1,82	2,73	3,64	4,55	5,46	6,37	7,28	8,19	9,10	10,01	10,92	11,83	12,74	13,66
220		0,90	1,79	2,69	3,59	4,48	5,38	6,28	7,18	8,07	8,97	9,87	10,76	11,66	12,56	13,45
230		0,88	1,77	2,65	3,54	4,42	5,30	6,19	7,07	7,96	8,84	9,72	10,61	11,49	12,38	13,26
240		0,87	1,74	2,61	3,49	4,36	5,23	6,10	6,97	7,84	8,71	9,58	10,46	11,33	12,20	13,07
250		0,86	1,72	2,58	3,44	4,30	5,15	6,01	6,87	7,73	8,59	9,45	10,31	11,17	12,03	12,89

Nitrogen

Gas flow in ml/min through column 0,32 mm/25 m pressure p in bar, T in °C

p		0,1	0,2	0,3	0,4	0,5	0,6	0,7	0,8	0,9	1	1,1	1,2	1,3	1,4	1,5
T																
20		0,63	1,27	1,90	2,54	3,17	3,80	4,44	5,07	5,70	6,34	6,97	7,61	8,24	8,87	9,51
30		0,62	1,24	1,86	2,48	3,10	3,72	4,34	4,96	5,58	6,20	6,82	7,45	8,07	8,69	9,31
40		0,61	1,22	1,82	2,43	3,04	3,65	4,25	4,86	5,47	6,08	6,68	7,29	7,90	8,51	9,11
50		0,60	1,19	1,79	2,38	2,98	3,57	4,17	4,76	5,36	5,95	6,55	7,14	7,74	8,33	8,93
60		0,58	1,17	1,75	2,33	2,92	3,50	4,08	4,67	5,25	5,83	6,42	7,00	7,58	8,17	8,75
70		0,57	1,14	1,72	2,29	2,86	3,43	4,00	4,58	5,15	5,72	6,29	6,86	7,44	8,01	8,58
80		0,56	1,12	1,68	2,24	2,80	3,37	3,93	4,49	5,05	5,61	6,17	6,73	7,29	7,85	8,41
90		0,55	1,10	1,65	2,20	2,75	3,30	3,85	4,40	4,95	5,50	6,05	6,61	7,16	7,71	8,26
100		0,54	1,08	1,62	2,16	2,70	3,24	3,78	4,32	4,86	5,40	5,94	6,48	7,02	7,56	8,10
110		0,53	1,06	1,59	2,12	2,65	3,18	3,71	4,24	4,77	5,30	5,84	6,37	6,90	7,43	7,96
120		0,52	1,04	1,56	2,08	2,61	3,13	3,65	4,17	4,69	5,21	5,73	6,25	6,77	7,29	7,82
130		0,51	1,02	1,54	2,05	2,56	3,07	3,58	4,10	4,61	5,12	5,63	6,14	6,66	7,17	7,68
140		0,50	1,01	1,51	2,01	2,52	3,02	3,52	4,03	4,53	5,03	5,53	6,04	6,54	7,04	7,55
150		0,49	0,99	1,48	1,98	2,47	2,97	3,46	3,96	4,45	4,95	5,44	5,94	6,43	6,92	7,42
160		0,49	0,97	1,46	1,95	2,43	2,92	3,40	3,89	4,38	4,86	5,35	5,84	6,32	6,81	7,30
170		0,48	0,96	1,44	1,91	2,39	2,87	3,35	3,83	4,31	4,78	5,26	5,74	6,22	6,70	7,18
180		0,47	0,94	1,41	1,88	2,35	2,82	3,30	3,77	4,24	4,71	5,18	5,65	6,12	6,59	7,06
190		0,46	0,93	1,39	1,85	2,32	2,78	3,24	3,71	4,17	4,63	5,10	5,56	6,02	6,49	6,95
200		0,46	0,91	1,37	1,82	2,28	2,74	3,19	3,65	4,10	4,56	5,02	5,47	5,93	6,39	6,84
210		0,45	0,90	1,35	1,80	2,25	2,69	3,14	3,59	4,04	4,49	4,94	5,39	5,84	6,29	6,74
220		0,44	0,88	1,33	1,77	2,21	2,65	3,10	3,54	3,98	4,42	4,87	5,31	5,75	6,19	6,63
230		0,44	0,87	1,31	1,74	2,18	2,61	3,05	3,49	3,92	4,36	4,79	5,23	5,66	6,10	6,54
240		0,43	0,86	1,29	1,72	2,15	2,58	3,01	3,43	3,86	4,29	4,72	5,15	5,58	6,01	6,44
250		0,42	0,85	1,27	1,69	2,12	2,54	2,96	3,39	3,81	4,23	4,65	5,08	5,50	5,92	6,35