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WHITE PAPER No. 38

Overcoming Challenges in Liquid Dispensing by Using the Right Accessories for Bottletop Dispensers

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Executive Summary

In this white paper we give some detailed examples of the beneficial use of the correct accessory with bottletop dispensers and show the possibilities beyond simple single stroke dispensing. Bottletop dispensers are used for multiple applications in the laboratory. Special materials must be used for parts getting in direct liquid contact to ensure high chemical resistance against aggressive solutions such as acids, bases and solvents. Additionally special adapters made from ETFE increase the chemical stability. Accessories such as a drying tube protect fuming acids from dilution or alkaline solutions from the formation of carbonates and can be easily attached to bottletop dispensers. The usage of a sterile filter on the bottletop dispenser ensures sterility of the medium in the bottle.

Figure 1: A bottletop dispenser with attached flexible discharge tube facilitates filling of small vessels or reagent tubes in a rack.

Introduction

In almost every laboratory worldwide challenging liquids such as acids, bases and solvents are used for various applications. Additionally some liquids need to be sterilized in advance and kept sterile during dispensing. In routine tasks where always the same volume of liquid is required, bottletop dispensers can be used to facilitate and accelerate work. If high numbers of samples - e.g. 50-150 samples - need to be prepared reproducibly without straining the lab employee the choice of the correct accessory for the bottletop dispenser can speed up the workflow and reduce risk of contamination. At the same time usage of less plastic consumables, such as tips, by direct dispensing out of the bottle comes with the benefit of protecting the environment and saving money.

With four exemplary applications with a high demand for throughput, sterility or requiring aggressive chemicals the beneficial usage of bottletop dispensers together with the correct accessory is shown.

Highly aggressive chemicals

Some chemicals such as acetonitrile used for HPLC and trace analysis are highly corrosive and can attack different plastic materials. This may lead to damage on a bottletop dispenser generating leakage, imprecise dispensing and in the worst case harming the user by corroded sharp edges. Additionally highly aggressive chemicals carry a health risk for the user when inhaled or in direct skin contact. Therefore bottletop dispensers need to be tight and screwed firmly on the bottle. But what if the adapter used for mounting the bottletop dispenser is attacked by the chemical?

Fuming acids and alkaline solutions

Fuming acids do not contain water and have the highest concentration possible. Some common examples are nitric acid or hydrochloric acid. Hydrochloric acid is highly aggressive with corrosive properties and often used in the chemical industry or in production of gelatin and other food additives or leather processing. To maintain the high concentration no water molecules shall get in contact with the acid, otherwise it would be diluted. Therefore arrangements are necessary to keep bottles containing fuming acids moisture-free.
Alkaline solutions such as potassium hydroxide or calcium carbonate are widely used for production of soaps, pharmaceuticals or cleaning products. These solutions tend to form carbonates when getting in contact to carbon dioxide of the surrounding air. Formation of carbonates leads to crystallization of the solution either in the bottle or in the tubes and piston of a bottletop dispenser. This inhibits working with the device unless it is cleaned properly. So how can one avoid dilution of a fuming acid, or carbonate formation in an alkaline solution?

Preparation of reagent tubes or small glass vessels

In microbiology reagent tubes are routinely prefilled with medium prior or after autoclaving. It is time consuming and typically hindered by tubes standing very close by in a rack. Often less than 5 mm space is between the tubes making it impossible to dispense medium into each tube without turning and tilting either the rack with tubes or the bottletop dispenser. But tilting the bottletop dispenser leads to inaccurate results and tilting the rack with tubes can lead to spillage.
For HPLC analysis small glass vials are used and sometimes prefilled with aggressive solvents. The vials have a small opening that is difficult to hit even with pipettes. Using a bottletop dispenser would offer great advantages in terms of filling speed and reproducibility. But often the dispensing tube of bottletop dispensers is inflexible and the opening too wide for small vessels. So are there any accessories facilitating filling small glass vessels and multiple reagent tubes?

Sterile liquids

Liquids are often pre-sterilized in bottles prior to usage. Especially in microbiology or cell culture applications sterility is a must. Working in a bio safety cabinet and constantly changing the consumable as well as fast working and closing the medium bottle after each use is necessary to keep the medium sterile. Using a bottletop dispenser for dispensing sterile medium is only possible if the device can be autoclaved while screwed on the bottle. But still all bottletop dispensers need a ventilation opening to facilitate liquid uptake while handling. And how is sterility guaranteed then?

Solutions & Benefits

Highly aggressive chemicals

Some parts of a bottletop dispenser come into direct contact with the dispensing liquid which can be acids, bases or (organic) solvents. Mainly the aspirating and discharge tube, the piston and cylinder, valves and valve heads are exposed to the liquid. These aggressive chemicals can lead to corrosion, plastic discoloration or melting, as well as general damage to multiple parts of a bottletop dispenser. Therefore these parts must be produced of chemically resistant materials such as borosilicate glass 3.3, ETFE/PTFE, PFA or Pt-Ir (see table 1 for abbreviations). Some parts which are not in direct contact to the liquid, as the bottle adapters, can be made of PP which is less resistant to chemicals. Nevertheless if a highly aggressive chemical such as acetonitrile, 98 % sulfuric acid or xylene is used small drops on the bottle neck may directly attack the adapter of the bottletop dispenser. This can lead to leakage, reduced suction by additional air intake through micro holes and reduced accuracy of the bottletop dispenser. One way to counter this effect is the usage of special chemically resistant bottle thread adapters made of ETFE. These adapters offer secure liquid dispensing of highly aggressive chemicals even up to 50 °C [1]. Eventually liquid drops at the bottle thread cannot harm the adapter and the above described consequences do not arise. Liquid dispensing is more reliable, reproducible and safe. For the Varispenser® 2 and 2x six ETFE adapters with the most common bottle thread sizes from 32 to 45 mm outer bottleneck diameter are available.

Table 1: Abbreviations of plastic material

Abbreviation	Chemical name
ETFE	ethylene/tetrafluoroethylene-copolymer
PFA	perfluoroalkoxy-copolymer
PP	polypropylene
PTFE	poly(tetrafluoroethylene)
Pt-Ir	platinum-iridium

Fuming acids and alkaline solutions

Adjusting the pH is daily routine in molecular biology labs, chemical industry and analytical labs. The higher concentrated an acid used for titration is, the faster is the pH adjustment since one needs only a few drops to change the pH value of the target solution. Therefore fuming acids can be used. The term “fuming acids” derives from gas rising off the surface of the liquid. The gas is introduced into acid to bind all residual water molecules, so that it is as concentrated as possible. A challenge is to keep the acid water free and thereby undiluted. But opening and closing a bottle or contact to surrounding air often leads to this dilution. Using a titration tool that is screwed onto the bottle containing a fuming acid is the first step to protect the liquid. Additionally arrangements to capture the moisture of the surrounding air need to be applied. This works effectively with using a drying tube filled with a moisture absorber (e.g. silica gel, calcium chloride) for moisture removal attached directly to the titration device. The Top Buret™ as well as the Varispenser models can be easily equipped with a drying tube by simply screwing it into the back opening [2] (Fig. 2). The liquid is protected and titration can be accomplished very precise.

A similar difficulty applies for alkaline solutions. The most common known alkaline solution is sodium hydroxide used for many different applications such as pH adjustment, production of cleaning agents and separation of DNA strands. Carbon dioxide of the surrounding air can lead to the formation of carbonates in alkaline solutions. This provokes crystallization and thereby blocking of the piston, changes in concentration of the liquid and inability to further dispense the liquid. To prevent these consequences the drying tube can be filled with e.g., potassium chloride or sodium hydroxide pills to remove carbon dioxide. This is possible with the Top Buret for titration applications or the Varispenser 2 and 2x which can be used for distinct dispensing of alkaline solutions in sample preparation or analysis.

Figure 2: Varispenser 2x with attached drying tube protects fuming acids from dilution.

Preparation of reagent tubes or small glass vessels
Culturing ammonium oxidizing bacteria is challenging and time consuming. These bacteria grow over a very long time at a slow growth rate until further experiments or phylogenetic studies can be done. These microorganisms grow in glass test tubes at 30 °C in special minimal media at low pH and addition of urea as sole substrate without antibiotics [3]. To reduce the risk of contamination during inoculation the test tubes with medium are prepared prior to autoclaving. This saves one autoclaving step in which the test tubes and medium would have been autoclaved separately. Additionally the step of transferring autoclaved sterile medium into the sterile tubes is also saved hence the tubes only have to be opened once during inoculation. To ensure the same volume of liquid in each test tube prior to autoclaving, bottletop dispensers are used to dispense the non-sterile medium into the tubes. But often the racks used for storage of the test tubes are very narrow and the tubes stand very close to each other. A typical canula arm and discharge tube of a bottletop dispenser is not long enough to reach over the whole rack to fill each tube. Additionally the discharge tube is often not long enough to reach deep into the test tubes.

A similar problem shows when preparing glass vessels for HPLC. Often aggressive solvents such as acetonitrile need to be prefilled into the vessels. Two difficulties are present then: a narrow opening of the glass vessel and an aggressive chemical. Using a pipette for transferring acetonitrile leads to dripping of the chemical out of the pipette tip because the air inside the pipette tip expands while the acetonitrile evaporates. This lowers accuracy and buries health risks by accidental drops on the lab bench. A bottletop dispenser decreases the health risk because the liquid is secured in the bottle and directly dispensed into the target vessel. Drop formation is highly reduced because the device functions according to the positive displacement principle without any air inside the piston or discharge tube. But still the discharge tube might be too big to easily hit the small glass vessels. Furthermore each tube needs to be held up directly under the discharge tube, this might carry health risks because the small vessel can slip from the fingers or if the opening is not hit, the liquid might flow over the hand or gloves. A more secure method is to keep the glass vessels in a rack and reach with the discharge tube into each vessel.

For this purpose a flexible discharge tube can be purchased as an accessory for the Varispenser 2 and 2x (Fig. 3). This flexible discharge tube has a length of 80 cm arranged in loops to save space while providing the highest flexibility and range possible to reach every test tube or small glass vessel in a rack [4]. The flexible discharge tube has a very fine tip that can go easily into small openings. Additionally the tip of the flexible discharge tube can be extended to reach deep into the test tubes.

Figure 3: The flexible discharge tube attached to the Varispenser 2 and 2x facilitates dispensing in small glass vessels or close-standing tubes.

Sterile liquids

In microbiology and cell culture sterile mediums are a must and either prepared directly in the lab or bought from suppliers. If the medium is prepared in bottles autoclaving is the preferred sterilization method. After that the medium needs to be dispensed into the reagent vessels. This is often accomplished by the usage of serological pipets and pipet helper. But every time the bottle is opened the medium is exposed to possible contaminants. Often medium is contaminated before the whole liquid could be used for experiments producing extra work autoclaving the contaminated medium and cleaning the bottles. Furthermore additional medium needs to be prepared in advance to be sure of having enough sterile medium on stock.

Using a bottletop dispenser that can be autoclaved directly on the bottle eliminates the first source of contamination of repeated opening and closing of the bottle and facilitates liquid dispensing. But still the bottletop dispenser together with the medium should be stored in the bio safety cabinet because each bottletop dispenser has an opening at the side or back through which surrounding air is introduced into the device. This is needed for proper function and facilitates uptake of liquid. If sterile medium is to be dispensed the introduction of surrounding air is unwanted and another source of contamination. To conquer this difficulty a filter adapter together with a sterile filter can be attached to the back of the Varispenser 2 and 2x (Fig. 4). The filter is tightly attached to the adapter and can easily be exchanged if necessary. The surrounding air is drawn through the filter with the desired pore size of 0.2 or 0.45 µM in each stroke eliminating most air contaminants such as dust or bacteria. Usage of a sterile filter protects the sterile liquid and increases safety when working with sensitive samples.

Figure 4: A sterile filter attached to the back of the Varispenser 2 and 2x secures sterility of the medium.

Summary

Bottletop dispensers are regarded as simple liquid handling tools to dispense defined liquid volumes into vessels. But used with the correct accessories bottletop dispensers can facilitate a broad range of applications and increase safety, speed and reliability of daily laboratory work. Highly aggressive chemicals need extra caution and can damage multiple parts on the bottletop dispenser and in the lab. The choice of materials used for the parts in direct liquid contact decides on the chemical stability of the tool and widens the usage in chemical industry. Additional accessory such as ETFE adapters increase the chemical stability even further to support relaxed handling of highly aggressive chemicals. In addition usage of a drying tube can protect fuming acids from dilution by air humidity. And alkaline solutions are protected from the formation of carbonates if carbon dioxide binding substances are filled into the drying tube.

Filling multiple reagent tubes or small glass vials for HPLC is facilitated by attaching a flexible discharge tube to the bottletop dispenser. This accessory enables the user to reach deep into each vessel, also the ones with small openings. Furthermore each vial in a rack can be reached without dangerous tilting of the bottletop dispenser and bottle. Last but not least the possibility of attaching a sterile filter to a bottletop dispenser offers a secure protective option to keep medium sterile also for longer times with repeated dispensing. So as you can see choosing the correct accessory for your bottletop dispenser saves time, work and protects you and your samples.

References

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[3] Koops HP, Purkhold U, Pommerening-Röser A, Timmermann G & Wagner M. “The Lithoautotrophic Ammonia-Oxidizing Bacteria.”. The Prokaryotes: An Evolving Electronic Resource for the Microbiological Community. 3rd edition, release 3.13. Hg. M. Dworkin et al. http://link.springer-ny.com/link/service/books/10125/. New York: Springer Verlag, 2003
[4] Operating Manual Varispenser® 2 and 2x (version 0040 000.143-00). www.eppendorf.com

Ordering information

Description	Order no. International	Order no. North America
Varispenser® 2 0.2 - 2 mL	4966 000.010	4966000010
Varispenser® 2 0.5 - 5 mL	4966 000.029	4966000029
Varispenser® 2 1 - 10 mL	4966 000.037	4966000037
Varispenser® 2 2.5 - 25 mL	4966 000.045	4966000045
Varispenser® 2 5 - 50 mL	4966 000.053	4966000053
Varispenser® 2 10 - 100 mL	4966 000.061	4966000061
Varispenser® 2x 0.2 - 2 mL	4967 000.014	4967000014
Varispenser® 2x 0.5 - 5 mL	4967 000.022	4967000022
Varispenser® 2x 1 - 10 mL	4967 000.030	4967000030
Varispenser® 2x 2.5 - 25 mL	4967 000.049	4967000049
Varispenser® 2x 5 - 50 mL	4967 000.057	4967000057
Varispenser® 2x 10 - 100 mL	4967 000.065	4967000065
Eppendorf Top Buret™ M	4965 000.017	4965000017
Eppendorf Top Buret™ H	4965 000.025	4965000025
Drying tube, without drying agent, for Eppendorf Top Buret™ and Varispenser® 2(x)	4966 509.002	4966509002
Flexible discharge tube, 80 cm, for Varispenser® 2(x), 2 mL, 5 mL, 10 mL	4966 501.001	4966501001
Flexible discharge tube, 80 cm, for Varispenser® 2(x), 25 mL, 50 mL, 100 mL	4966 502.008	4966502008
Ventilation screw with luer cone, PP, for microfilter, for Varispenser® 2(x)	4966 511.007	4966511007
Sterile membrane filter	We recommend using a standard sterile filter with a pore size of 0.2 or 0.45 µM with luer cone.	

Get your demo of the Varispenser® 2, 2x or the Eppendorf Top Buret™ to convince yourself of the simplification of your application. Contact your local sales rep for further information.

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