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Short communication

# Evaluation of a rinsing-based cleaning process for pipes

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**Abstract**

Pipes of various designs were constructed. Pipes were filled with a model solution resembling a dermal solution product. After the removal of the model solution, pipes were rinsed several times with ethanol and rinsing solutions of each step analyzed by gas chromatography. The results gave the information about the dependency between the configuration of the pipe and the efficiency of the cleaning operation. From concentrations measured in the reactor, expected concentrations in rinsing solutions from pipes were predicted. The obtained results confirm that the amount of residues per surface area increases when a pipe includes bends and valves. In terms of extra contamination, each bend was equal to 25 cm, while each valve was equal to 100 cm of pipe length when pipes of 1.8 cm in diameter were used. It was proven that the contributions of individual valves and bends in the pipe are additive in the calculation. The validity of the proposed model was confirmed by experimental data.


*Keywords:* Cleaning validation; Rinsing; Gas chromatography; Pipe; Valve

## 1. Introduction

A number of products have been recalled from the market due to cross-contamination with pharmaceuticals and chemicals in recent years. In pharmaceutical industry and more often in food industry, the removal of possible residues from the surfaces of production equipment is becoming a very important requirement. In most production lines the same equipment is used for processing different products. Effective cleaning procedures are thus needed in order to avoid contamination of subsequent products. According to regulatory requirements, equipment must be clean and cleaning must be documented [1–3]. Cleaning operations should be validated, with all steps carefully documented, from preparation of a validation plan through the final report. Selected analytical methods must be sensitive, selective and robust. The most important factors in measurements of effectiveness of reliable cleaning are a standardized sampling procedure and a consistent recovery [4–8].

There are two general types of sampling: direct surface sampling using swabbing methods and the use of rinsing solutions. Swabbing is more popular because it is a direct way of sampling. It gives the level of contamination per selected surface area, and even insoluble residues can be sampled by physical removal. Unfortunately many systems in production lines cannot be routinely dissembled and in this case the rinsing method has to be used. In this case a certain amount of insoluble or physically occluded residues should be taken into account.

An important key to effective cleaning is also a scientifically justified analytical limit. Limits of surface contamination are usually calculated from the acceptance limits in the subsequently manufactured drug product on the same production line. For practical work a helpful document is FDA's guidance for determining residue limits [1]. This document was prepared by FDA experts from industry on a logical, practicable and verifiable basis. It proposes that the equipment must be visually clean, with a maximum carryover level of 10 ppm to the subsequent product as a general rule. The tolerance level for an active agent is 1/1000 of the minimum daily dose from previous product in the maximum daily dose

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of the subsequent product. There are also other traceable and understandable calculations of limits [9–11], but in the presented paper the simplest mode to determine the range and limits of applied analytical procedures was selected.

## 2. Experimental

An experiment was prepared in order to obtain the correlation between the shape of a pipe and the efficiency of the cleaning process. Test equipment was constructed from stainless steel seamless pipes with internal diameter of 1.8 cm, the same type used as in the selected production line. The valves used were similar although not completely identical to those in the production line. Three typical types of model pipes have been built (Fig. 1). The inner volume of all pipes tested was approximately the same, regardless of the shape, as deduced from calculations and measurements. Pipes were filled with model solutions resembling real products. After 24 h the solutions were removed and pipes were rinsed several times with ethanol. The rinsing solutions were analyzed with an in-laboratory developed and validated high-throughput GC method. The results gave the information about the dependency between the form of the pipe and the efficiency of the cleaning operation.

### 2.1. Gas chromatography

The relatively simple composition of the dermal solution allowed the development and validation of a fast and simple capillary GC method for simultaneous determination of camphor and menthol in both the product and pipe rinsing solutions. Thus, no issues regarding sample matrix were encountered such as in a method published by Mirza and Tan [12].

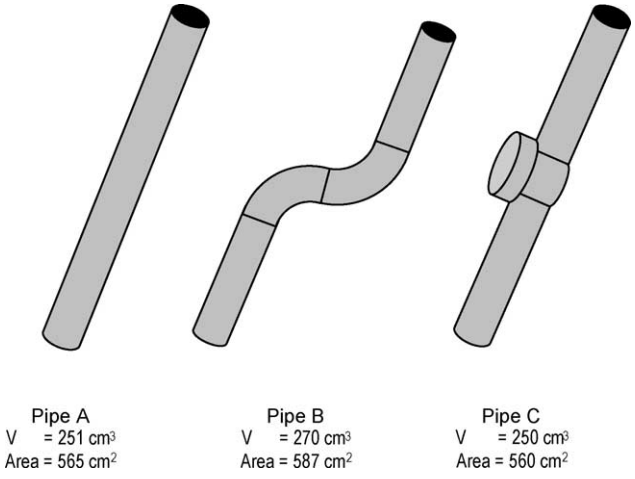


Fig. 1. Different forms of test pipes; volumes and areas were calculated from their inner dimensions. Exact volumes were measured gravimetrically using water.

Table 1
GC conditions

| | |
|---|---|
| Carrier gas | Helium |
| Flow mode | Constant flow, 12 mL/min |
| Injection volume | 0.5 μL |
| Split ratio | 1:15 |
| Inlet temperature | 250 °C |
| Detector temperature | 250 °C |
| Make up gas | Helium, 25 mL/min |
| GC column | Optima 5 (Macherey–Nagel), 25 m × 0.32 mm, 0.25 μm film thickness |
| Oven temperature program | 80 °C (0.8 min) to 125 °C (0.5 min) at 45 °C/min |
| GC run time | 2.3 min |

All solvents used in preparation of standards and samples were HPLC grade, menthol, camphor, benzyl alcohol (used as internal standard, IS) were >99.5% pure. All chemicals were purchased from Merck (Darmstadt, Germany).

Samples were analyzed with a Finnigan Focus GC system equipped with a flame ionization detector (FID) and an autosampler AI3000 for liquid samples (Thermo Electron Corporation, Rodano Milan, Italy). The chromatographic conditions are shown in Table 1. Quantification of separated peaks was performed with ChromCard v. 2.0 (Thermo Electron Corporation) data acquisition and processing software. Chromatograms of standard and rinsing solutions are shown in Fig. 2.

### 2.2. Preparation of solutions

#### 2.2.1. Preparation of standards

Stock solutions of menthol (1 mg/mL), camphor (1 mg/mL), and benzyl alcohol (0.25 mg/mL) were prepared. Standard solutions for calibration were prepared daily, meanwhile two sets of QC samples at 0.05 and 0.005 mg/mL were prepared at the beginning, by appropriate dilution of stock solutions. Concentrations of calibration standards for both menthol and camphor were 0.5, 0.1, 0.02, 0.005 and 0.001 mg/mL. The concentration of IS was 0.05 mg/mL.

#### 2.2.2. Preparation of samples

Samples for analysis were prepared by rinsing the model pipes. Prepared model pipes were filled with a model solution containing 0.5% (w/v) of both menthol and camphor, and closed with plugs at both ends. Pipes were placed on a stand and slowly rotated for 24 h with an appropriate device. Model solution was then removed and the pipes were rinsed five times with ethanol. Each time, 50 mL of ethanol was applied into the test pipe and the latter was then securely plugged at both ends. Ten inversions of the pipe followed, ensuring an effective wetting of the whole inner surface with ethanol. After that the obtained solution was removed from the pipe. Samples were stored in dark bottles at 4 °C. Prior to GC analysis, IS solution was added in appropriate amount to reach the concentration of 0.05 mg/mL as in standard solutions.

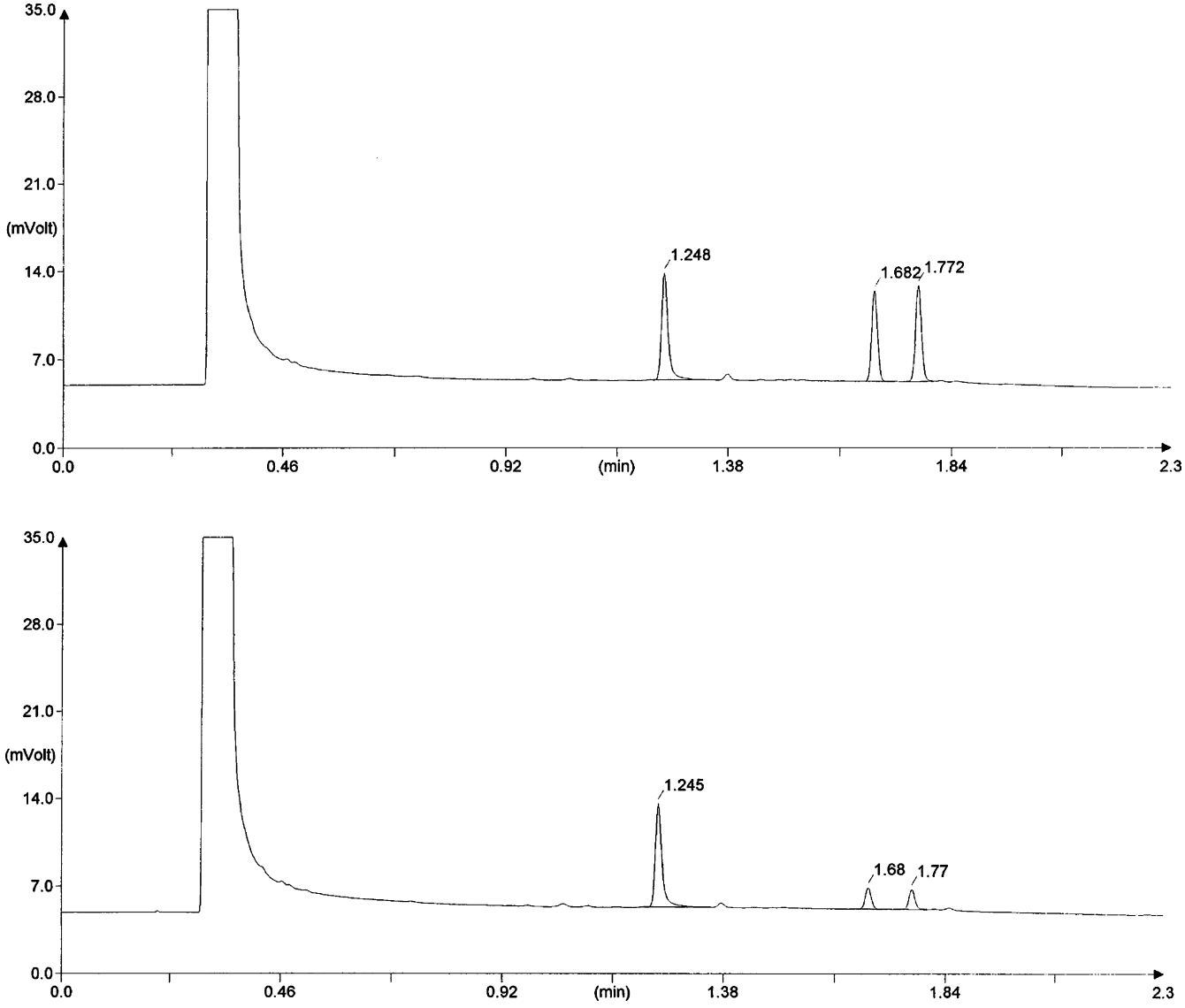


Fig. 2. GC chromatograms of standard (above) and rinsing solution (below). The peaks shown are benzyl alcohol (internal standard), menthol and camphor in the order of elution (1.25, 1.68 and 1.77 min, respectively).

### 2.3. GC method validation

The GC method was validated according to the prepared validation protocol. Selected acceptance criteria and results of obtained measurements are shown in Table 2. System suitability test was performed as a leading sequence in all experiments by injecting five replicates of standard solution with concentration of 0.05 mg/mL. Selectivity of the developed method was adequate, since no interfering peaks were present in the chromatograms of standard and sample solutions, as well as in blank injections. Precision, intermediate precision and stability were determined from sets of calibration solutions and QC samples. In three consecutive days calibration curves in triplicate and six replicates of QC samples at each concentration were injected.

Stock solutions, working standards and rinsing solutions were stored in a refrigerator at 4 °C. For stability test at room temperature a set of QC samples was stored at room temperature for additional 24 h (22 °C) and re-analyzed. The obtained values were evaluated on the basis of comparison of the results from freshly prepared calibration curves and QC samples stored in the refrigerator (4 °C). Linearity range, limits of detection (LOD) and limits of quantitation (LOQ) were evaluated from calibration curves with five calibration standards from 0.001 to 0.5 mg/mL. Values of slope ($b$), intercept ($a$), correlation coefficient ($R$), and standard deviation of slope $V(b)$ were calculated using weighted calibration curves. Weight factors were calculated from the relationship $fw_i = 1/(100 + 50X_i)^2$. LOD and LOQ were calculated from the confidence interval.

Table 2
Validation parameters, acceptance criteria and obtained results of the GC method

| Parameters | Acceptance criteria | Results and remarks | | |
|---|---|---|---|---|
| System suitability test | Peak area vs. IS area better than 5% R.S.D. ($n = 5$, 50 μg/mL)<br>Rt better than 0.5% R.S.D. ($n = 5$, 50 μg/mL) | Menthol<br>Camphor | 0.3% (Area vs. IS)<br>0.5% (Area vs. IS) | 0.1% (Rt)[a]<br>0.1% (Rt)[a] |
| Selectivity | $R > 2$[b] | Menthol/camphor | 2.3 | |
| Precision (from QC) | <5% R.S.D. ($n = 6$) | Menthol<br>Camphor | 0.5% (5 μg/mL)<br>0.5% (5 μg/mL) | 0.4% (50 μg/mL)<br>0.7% (50 μg/mL) |
| Accuracy (from QC) | 100 ± 5% ($n = 6$) | Menthol<br>Camphor | 101.4% (5 μg/mL)<br>100.2% (5 μg/mL) | 100.9% (50 μg/mL)<br>102.9% (50 μg/mL) |
| Linearity and range | $r > 0.999$ (1–500 μg/L) | Menthol<br>Camphor | $r = 0.9996$[c]<br>$r = 0.9998$[c] | |
| LOD | From confidence interval | Menthol<br>Camphor | 0.2 μg/mL<br>0.4 μg/mL | |
| LOQ | From confidence interval | Menthol<br>Camphor | 0.3 μg/mL<br>0.6 μg/mL | |
| Stability | 100 ± 10%, 24 h at room temperature | Menthol<br>Camphor | 101.5% (5 μg/mL)<br>96.8% (5 μg/mL) | 101.2% (50 μg/mL)<br>99.5% (50 μg/mL) |

[a] Retention time.
[b] Resolution.
[c] Weighted calibration curves.

### 2.4. Acceptance limits

The acceptance limit in this experiment was calculated from the results obtained from a real production line, illustrated in Fig. 3. In the selected production line dermal solutions are constantly produced. The basic differences between solutions are active substances, their concentrations and volumes of products. A typical batch of product consists of 1000 L from which 3000 to 5000 bottles are prepared.

To calculate the limit ($L_1$) of the active agent in a subsequently manufactured product the minimum daily dose of product A and the maximum daily dose of subsequent product B were taken into account. In the example discussed, a dermal solution (product A) has an active level of 0.5% and is used three times a day in 5 mL doses. The calculated minimum daily dose is 75 mg. If the product B is a similar product and is used five times daily also in 5 mL doses, the residue limit in the subsequent product can be calculated according to Eq. (1). The selected safety factor is 0.001, as mentioned.

$$L_1 = \frac{0.001 \times 75\,\text{mg/day}}{25\,\text{mL/day}} = 0.003\,\text{mg/mL} \quad (1)$$

The results of this calculation can be compared with suggested values of 0.010 mg/mL from other criteria (FDA, Lilly, etc.). For dermal solutions it is not necessary to use a more stringent safety factor in order to select and justify the limit. Once the residue limit in the subsequent product B is determined the residue limit in terms of contamination level per surface area ($L_2$) could be calculated (Eq. (2)).

$$L_2 = \frac{L_1 \times B}{E \times f} \quad (2)$$

where $B$ is the batch size of product B (kg or L), $E$ the equipment area ($cm^2$), $f$ the units conversion factor.

Areas and volumes of individual parts of equipment have been estimated. The entire area is about 50,000 $cm^2$, and the area of pipes and valves is about 5000 $cm^2$. The total volume of the equipment is about 1,200,000 $cm^3$, while the volume of pipes and valves is only 6000 $cm^3$. Assuming an approximately equal distribution of residuals on pipe and reactor surfaces, a more than 20-fold difference in volume–area ratios between pipe and reactor should not be neglected, when calculating the acceptance limit of the rinsing solution. In such a case the calculated contamination level $L_2$ (Eq. (2)) for whole equipment is 51 μg/$cm^2$. If the reactor is washed with solvent whose volume is equal to 0.1% of the reactor vol-

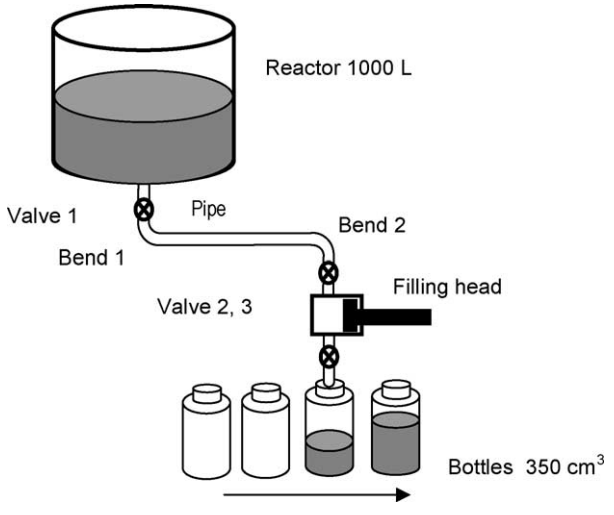


Fig. 3. Schematic presentation of a production line, constructed from a reactor, a pipe, and a filling head. In the calculation procedure three valves and three bends (one for filling head) were considered.

Table 3
Concentrations determined in rinsing solutions from test pipes

| Rinsing | Straight pipe (μg/mL) | | Pipe with valve (μg/mL) | | Pipe with two bends (μg/mL) | |
|---|---|---|---|---|---|---|
| | Menthol | Camphor | Menthol | Camphor | Menthol | Camphor |
| 1 | 124.4 | 123.6 | 252.8 | 253.1 | 190.1 | 189.1 |
| 2 | 2.7 | 3.1 | 45.3 | 46.3 | 20.3 | 4.3 |
| 3 | <0.3 | <0.6 | 2.2 | 3.0 | <0.3 | <0.6 |
| 4 | <0.3 | <0.6 | 2.1 | 2.7 | <0.3 | <0.6 |
| 5 | <0.3 | <0.6 | 0.6 | <0.6 | <0.3 | <0.6 |
| Total amount | | | | | | |
| μg/mL[a] | 25.4 | 25.3 | 60.6 | 61.0 | 42.1 | 38.7 |
| mg/pipe | 6.36 | 6.34 | 15.15 | 15.26 | 10.52 | 9.67 |
| μg/cm$^2$ | 11.2 | 11.2 | 27.1 | 27.2 | 17.9 | 16.5 |
| Steps | | | | | | |
| 1 | 97.9 | 97.09 | 198.9 | 199.8 | 149.6 | 149.3 |
| 2 | 2.1 | 2.4 | 35.6 | 36.5 | 16.0 | 3.4 |
| 3 | 0.0 | 0.0 | 1.7 | 2.4 | 0.0 | 0.0 |
| 4 | 0.0 | 0.0 | 1.7 | 2.1 | 0.0 | 0.0 |
| 5 | 0.0 | 0.0 | 0.5 | 0.0 | 0.0 | 0.0 |
| %[b] | 100.0 | 100.0 | 238.4 | 240.8 | 165.5 | 152.6 |

[a] Cumulative concentration determined from all rinsing steps; each pipe was rinsed five times with 50 mL of ethanol.
[b] Relative amount in %, compared to the value of the straight pipe.

ume the concentration in the rinsing solution is 1.9 mg/mL. If the pipe is washed with solvent which volume equals to 1.0% of the pipe the concentration in the rinsing solution is 4.3 mg/mL. More than two-fold higher concentrations are thus expected in the latter case.

## 3. Results

The results confirm that the amount of residuals increases when a pipe includes bends and/or valves. Measured values show the ratio between the amount of residuals in different pipe configurations and also how fast the elution of a substance from the pipe is.

For comparison, the pipe with two bends (C) has 50–60% higher amount of deposits, but their elution is practically the same as in the straight pipe (A). 90–97% of deposits are removed with the first rinsing. When pipes include valves, the situation is different. The amount of deposits is significantly higher and their majority cannot be eluted in one step. The first rinsing removes only 83% of deposits and after the second one there is still about 3% of deposits. Detailed values are shown in Table 3 and depicted in Fig. 4. Some differences between the behavior of menthol and camphor were observed, but they are not significant.

The obtained results were helpful for choosing the right approach to calculate the approximate concentrations of contaminants in rinsing solutions when the size and the configuration of the pipe, and the concentration of contaminants (obtained by swabbing the reactor surface) are known. The expected concentrations in rinsing solutions were estimated based on determined surface area contamination and pipe corrected inner area. The inner pipe area was virtually increased so that for each bend or valve 25 or 140 cm were added to the total length of the 1.8 cm pipe, respectively. Concentrations were calculated according to Eq. (3).

$$c = \frac{2\pi r_p}{V_r}(l_p + f_b n_b + f_v n_v)c_{swb} \quad (3)$$

where $c$ is the concentration of the rinsing solution (mg/L), $c_{swb}$ the concentration obtained by swabbing (μg/cm$^2$), $f_b$ the bend factor (cm), $f_v$ the valve factor (cm), $n_b$ the number of bends in the pipe, $n_v$ the number of valves in the pipe, $l_p$ the

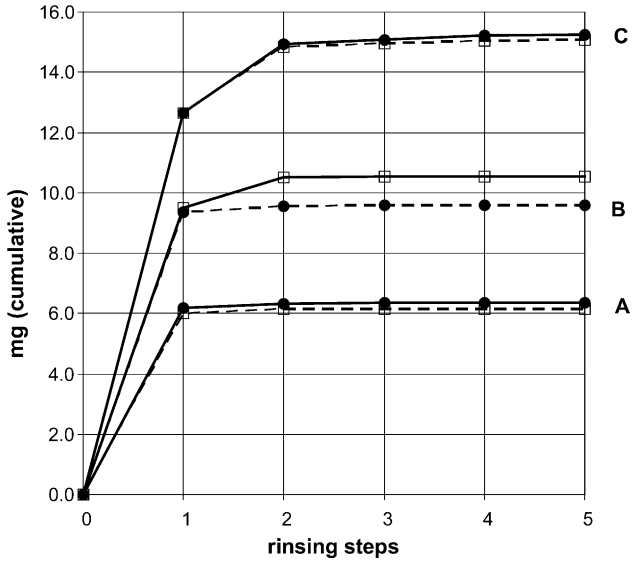


Fig. 4. Schematic representation of cumulative amounts of menthol (empty squares) and camphor (black circles) after five rinsing steps. Curves are marked with A for the straight pipe, with B for the bent pipe, and with C for the pipe with valve.

Table 4
Calculated and measured cumulative amounts obtained by rinsing a production line

| Rinsing volume (L) | Menthol | | Camphor | |
|---|---|---|---|---|
| | Measured values (mg) | Calculated values (mg) | Measured values (mg) | Calculated values (mg) |
| 0.300 | 20.1 | 28.9 | 9.1 | 12.2 |
| 0.600 | 25.5 | 31.3 | 11.2 | 13.2 |
| 0.900 | 27.5 | 31.5 | 12.0 | 13.3 |
| 1.200 | 29.1 | 31.6 | 12.4 | 13.3 |
| 1.500 | 30.4 | 31.7 | 12.8 | 13.4 |

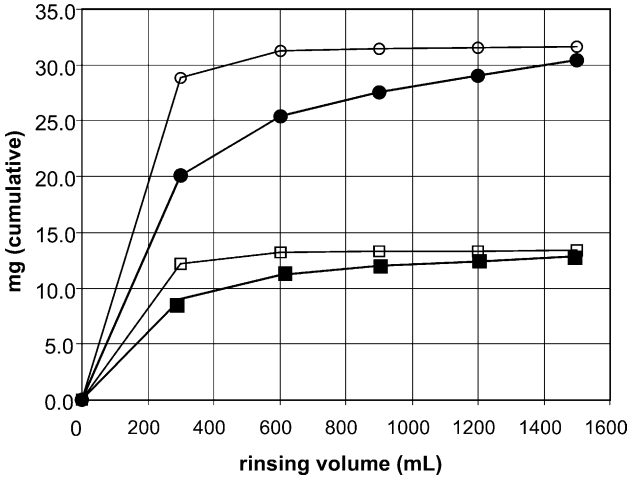


Fig. 5. Diagram showing the calculated cumulative amount of rinsed camphor (empty squares) and menthol (empty circles); calculation is based on measured concentrations of residual camphor $c = 1.9\ \mu g/cm^2$ and menthol $c = 4.5\ \mu g/cm^2$ on reactor surface obtained with swabbing. The curves with black circles (menthol) and black squares (camphor) represent the results obtained from the rinsing of the manufacturing equipment, which includes three valves and three bends.

pipe length (cm), $V_r$ the rinsing solution volume ($cm^3$), and $r_p$ the pipe radius (cm)

Calculated values according to Eq. (3) were compared with experimentally obtained concentrations from a production line. Due to the differences between valves from the production line and model pipes, the valve factor of 100 cm was used, instead of 140 cm, which gave a good agreement between calculated and measured values. More importantly, when factors from Table 3 were used, concentrations up to the fifth rinsing step in the production line were predicted, regardless of type of valve used. The results are shown in Table 4 and Fig. 5.

## 4. Conclusion

In many cases, pipes are not treated with enough care. Easily accessible areas are thoroughly examined, but at the same time a poorly cleaned pipe as the major contribution of contaminants to the final product is not considered, especially at the beginning of the production of the subsequent product. Contaminants are deposited on a large area with complicated sections and are later dissolved in a relatively small volume of rinsing solution. Obviously, contaminants in not properly cleaned pipes could spoil a starting batch of the product. Experimental results evidence how important is the prediction (and validation) and the control of pipe cleaning procedures as well. Volume–area ratio in a pipe is smaller compared to other parts of the equipment. In the case presented, the ratio in the reactor was more than 20 times higher than in the pipe. Consequently, the concentrations in rinsing solutions could be very high.

Experimental results show that the prepared formula in which bends and valves are replaced with adequate lengths of pipe allow the prediction of the concentrations in rinsing solutions, and that the individual sections are additive in the calculation. The inner pipe area is calculated from the corrected length; meanwhile its volume is calculated from the original dimensions.

The obtained results are not universal. For each production line or plant, a new model should be constructed from the elements that are actually built in the production line. With such model we get parameters, which help us to prepare and correctly evaluate cleaning process in our plants.

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