

# A method for quantification of formaldehyde in the presence of formaldehyde donors in skin-care products

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Reliable and uncomplicated methods for detection of free formaldehyde in products preserved with formaldehyde donors are desirable to decrease the risk of allergic contact dermatitis. The aim of this study was to develop a method that could be used in clinics and workplaces for quantification of free formaldehyde in products preserved with formaldehyde donors. The method developed is named the closed container diffusion (CCD) method. Formaldehyde in a sample is allowed to evaporate in a closed container and react with 2,4-dinitrophenylhydrazine coated on a glass fibre filter. The hydrazone formed is analyzed with HPLC. The method was tested on 3 different formaldehyde donors, imidazolidinyl urea, diazolidinyl urea and 2-bromo-2-nitropropane-1,3-diol, using 4 different cream bases. The results obtained with this method accord with those obtained with the official method within the European Union (EU). The method is sensitive enough for analysis of patients' products and for control of labelled amounts of formaldehyde in technical products without solvent extraction. As a result of our studies, we observed a risk of exceeding the labelling limit for free formaldehyde in cosmetic products when using the highest amount of diazolidinyl urea allowed within the EU.

**Key words:** 2-bromo-2-nitropropane-1,3-diol; Bronopol; closed container diffusion (CCD) method; cream base; diazolidinyl urea; formaldehyde; formaldehyde donors; Germall 115; Germall II; imidazolidinyl urea; preservatives; quantification of free formaldehyde. © Munksgaard, 1998.

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Imidazolidinyl urea (Fig 1:1) and diazolidinyl urea (Fig 1:2) are 2 of the most common preservatives in the group of formaldehyde donors used in skin care products (1). From each molecule of imidazolidinyl urea and diazolidinyl urea, 2 and 4 formaldehyde molecules, respectively, can be released. Formaldehyde is a well-known contact allergen (2, 3). When patch testing dermatitis patients, concomitant reactions to formaldehyde and formaldehyde donors are obtained, indicating that the formaldehyde formed from the donors causes allergic reactions (4). However, cases of contact allergy to imidazolidinyl urea and diazolidinyl urea themselves are also described (4–6).

Reliable and uncomplicated methods for detection of free formaldehyde in products preserved with formaldehyde donors are desirable to decrease the risk of allergic contact dermatitis. Different methods for detection of formaldehyde are

compared in the literature (7). Quantification of free formaldehyde in the presence of formaldehyde donors is problematic. With the commonly applied methods, the donors will degrade in the presence of the reagent, and formaldehyde is released continuously during the analysis. This usually gives too high and non-reproducible values. In the method adopted within the European Union (EU) (8), the quantification of free formaldehyde is based on condensation of free formaldehyde with ammonium acetate and acetylacetone (pentane-2,4-dione) to form fluorescent 3,5-diacetyl-1,4-dihydrolutidine (the "acetylacetone method"). In the presence of formaldehyde donors, high performance liquid chromatography (HPLC) with post-column derivatization is used to separate free formaldehyde from the donor (9). However, this method is too complicated to be used in clinical practice and at different workplaces.

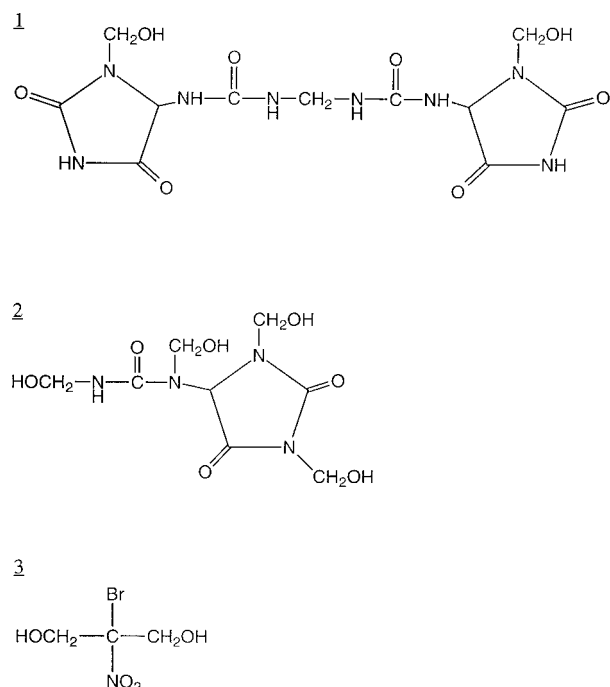


Fig. 1. Imidazolidinyl urea 1, diazolidinyl urea 2, and Bronopol 3.

Quantification of formaldehyde after derivatization with 2,4-dinitrophenylhydrazine (2,4-DNPH) is another common and reliable HPLC method (10). This method is sensitive, but the formaldehyde donors are also degraded with this method, which makes it less suitable for detection of free formaldehyde when formaldehyde donors are present in the formula.

The indirect chromotropic acid method (11) is a simple qualitative method that is commonly used in dermatological clinics. The analysis is based on the violet colour formed at the reaction between free formaldehyde evaporating from the product and chromotropic acid. However, other aldehydes and ketones will give colours that interfere with the colour deriving from formaldehyde (7, 12).

The aim of the present study was to develop a method that could be used in clinics and workplaces for quantification of free formaldehyde in products preserved with formaldehyde donors. We wanted to combine the simplicity of the chromotropic acid method with the reliability of the HPLC method with 2,4-DNPH derivatization. A method for quantification of formaldehyde in air in which free formaldehyde reacted with 2,4-DNPH-coated glass fibre filters (13) served as a starting point in our work. The formaldehyde donors used in this methodological study were imidazolidinyl urea, diazolidinyl urea and 2-bromo-2-nitropropane-1,3-diol (Bronopol) (Fig. 1:3). Cream bases with different

viscosity were chosen, since the viscosity may affect the release of formaldehyde.

## Materials and Methods

### Chemicals and cream bases

Formaldehyde stabilized with 10% methanol was obtained from Riedel-de Haen. The content of formaldehyde was determined iodometrically to 41.1% (8). Imidazolidinyl urea (CAS RN 39236-46-9, purity 94%), diazolidinyl urea (CAS RN 78491-02-8, purity >99%), and Bronopol (2-bromo-2-nitropropane-1,3-diol, Bronopol®, CAS RN 52-51-7, purity 99.9%) were obtained from Sigma Chemical Co. The purity stated by the producers was not verified. Acetonitrile *p. a.* containing <0.01 µg/g of free formaldehyde according to HPLC analysis (10, 13) was obtained from Merck. 2,4-DNPH (Fluka, *p. a.*), recrystallized 2× in 4 M HCl, was used in the synthesis of formaldehyde 2,4-dinitrophenylhydrazone. The formaldehyde hydrazone was kept in the refrigerator until used for standard solutions.

3 commercially available cream bases were included in the study. The products were o/w emulsions used in skin care products. In addition, Essex cream was diluted with water, by stirring in a mortar, to obtain a cream base with low viscosity and the same pH value as the original cream base. The main ingredients, the viscosity, and the pH values of the different cream bases are given in Table 1. The viscosity was determined using a CarriMed CSL Rheomatic instrument with a measurement system consisting of a cone and a plate. The experimental mode was shear stress sweep, 3 to 100 s<sup>-1</sup> for 2 min.

### Quantification of formaldehyde with the closed container diffusion (CCD) method

A sample to be analyzed was placed in a special glass vial with a holder for a glass fibre filter coated with 2,4-DNPH. The glass vial was put in an air-tight glass-stoppered glass jar (Fig. 2). Free formaldehyde evaporating from the sample reacted with 2,4-DNPH on the filter. To extract the hydrazone, the filter was put in an Erlenmeyer flask, acetonitrile (3 ml) was added and the flask was shaken for 10 min. The content of formaldehyde hydrazone in the solution was determined using HPLC (10, 13).

The instruments used in the HPLC analyses were an LKB-2150 pump, a Shimadzu SPD-6A variable UV-detector, and an autoinjector CMA/200 from Carnegie Medicine, Stockholm, Sweden. Data was evaluated using Dynamax HPLC Method Manager from Rainin Instrumental Com-

Table 1. Cream bases in the study

| Product                                       | Major ingredients                                                                                                                                    | o/w emulsion | pH  | Viscosity at 100 <sup>s-1</sup> (mPas) |
|-----------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------|--------------|-----|----------------------------------------|
| Locobase <sup>a</sup><br>(Brocades Pharma)    | stearyl- and cetylalcohol<br>cetomacrogol 1000<br>liquid paraffin<br>petrolatum<br>citric buffer<br>water                                            | 70/30        | 3.8 | 5262                                   |
| Essex cream <sup>b</sup><br>(Shering-Plough)  | cetomacrogol 1000<br>stearyl- and cetylalcohol<br>liquid paraffin<br>petrolatum<br>phosphoric buffer<br>water                                        | 28/72        | 4.7 | 1400                                   |
| Essex cream diluted <sup>c</sup>              |                                                                                                                                                      | 11/89        | 4.4 | 230                                    |
| Essex lotion <sup>a</sup><br>(Shering-Plough) | carbomer 941<br>starch<br>glycerol<br>petrolatum<br>cetylalcohol<br>stearic- and palmitric acid<br>glycerylmonostearate<br>sodium hydroxide<br>water | 9/91         | 6.0 | 490                                    |

<sup>a</sup>No preservatives. <sup>b</sup>Preserved with chlorocresol 0.1%. <sup>c</sup>Essex cream diluted with water at our laboratory.

pany, Inc, Woburn, Ma, USA. The analyses were performed on a CT-sil ODS column (C18-silica, 250×4.6 mm, 5  $\mu$ m) from ChromTech AB, Stockholm, Sweden. The mobile phase was 60% acetonitrile and 40% water, with a flow rate of 1 ml/min. The injected volume was 10  $\mu$ l. The detector was set at 350 nm. Each analysis was performed 2×.

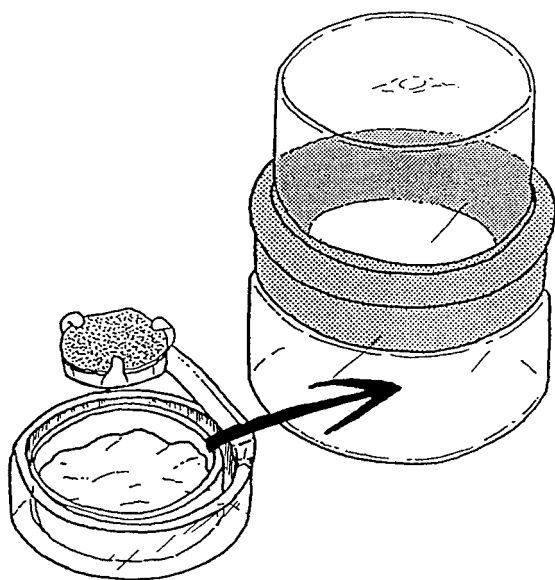


Fig. 2. Equipment for the closed container diffusion (CCD) method.

The detection limit for the system was 1.4 ng/g formaldehyde (S/N=3). Quantification was performed using synthesized formaldehyde-hydrazone as reference compound (10, 13). The amount of formaldehyde was calculated from the calibration curve. The calibration curve was linear ( $r=0.999$ ) in the range 0–25  $\mu$ g/g formaldehyde. When needed, the solution of hydrazone from the filter was diluted with additional acetonitrile to a concentration within the linear range.

To prepare the 2,4-DNPH covered filters, 300 mg 2,4-DNPH was dissolved in a solution of 0.5 ml 85% phosphoric acid ( $H_3PO_4$ ), 1.5 ml 20% glycerol in ethanol and 9 ml acetonitrile. A 13 mm glass fibre filter from SKC Inc, Eighty four, Pa, USA was soaked in the solution and dried in air at room temperature for 30 min. About 2 mg of 2,4-DNPH was coated on each filter. The hydrazone filters can be kept in the freezer at  $-18^\circ C$  for 4 months without deterioration (13). To determine the background value of non-exposed filters, 10 filters were prepared and analyzed for content of formaldehyde. A mean value of 0.02  $\mu$ g formaldehyde per filter was found. The standard deviation was 0.014.  $0.02 \mu g + 3 \times 0.014 = 0.06 \mu g$  per filter was chosen as the detection limit.

The sample to be analyzed was placed in the closed container immediately after addition of formaldehyde or formaldehyde donor. To obtain an optimal exposure time, free formaldehyde on the filter was determined from 1 to 48 h using 1.0 g of a formaldehyde solution (41  $\mu$ g/g in water). A tem-

perature of 35°C was chosen to mimic the skin temperature. The closed containers were kept dark during the experiment. A linear correlation ( $r=0.996$ ) between time and formaldehyde detected on the filter was observed up to 23.5 h. An exposure time of 18 h was chosen for the further methodological experiments, since the amount of formaldehyde reacting on the filter was fairly high after 18 h and it was found convenient from a practical point of view.

**Formaldehyde in water.** The reproducibility of the method was investigated. Free formaldehyde reacting on the filter was determined for each of 10 samples of 1.0 g water solutions with a concentration of 50 µg/g formaldehyde. To investigate the linearity, free formaldehyde reacting on the filter was determined for water solutions of formaldehyde in known concentrations from 0–1000 µg/g. In one series we used samples of 1.0 g and in another samples of 0.5 g.

**Formaldehyde in cream bases.** Formaldehyde was added to 0.5 g of Locobase, Essex cream, Essex cream dil. and Essex lotion, respectively, so that series with known concentrations (0–400 µg/g) were obtained. Formaldehyde reacting on the filter was analyzed. The same series, but with samples of 1.0 g, were also studied.

**Quantification of free formaldehyde from formaldehyde donors.** To investigate the reproducibility of the method using formaldehyde donors in cream bases, the content of free formaldehyde was determined for 10 samples of 1.0 g of Essex Cream containing 100 µg/g of diazolidinyl urea, for 10 samples of 0.5 g of Essex cream dil. containing 200 µg/g of imidazolidinyl urea, and for 10 samples of 0.5 g Essex lotion containing 200 µg/g of Bronopol. Furthermore, formaldehyde on the filter was analyzed for known concentrations from 0 to 3000 µg/g of diazolidinyl urea or imidazolidinyl urea in 0.5 g Essex cream dil., and for Bronopol (0–3000 µg/g) in 0.5 g Essex lotion. The ratio between formaldehyde detected and formaldehyde added to the cream base in the experiment described above was used to quantify free formaldehyde in the cream base.

#### *Quantification of formaldehyde in the presence of formaldehyde donors – the official method within EU*

The instruments used were ConstaMetric pumps from LDC Analytical, a Kratos Spectroflow 757 UV detector from Applied Biosystem, a CMA 200/240 autoinjector from Carnegie Medicine, Stockholm, Sweden, and a column heater from CROCO CIL, Cluzeau-Info labo B.P., Sainte-Fou-La Grande, France. Data were evaluated using PeakPro Chromatography Data System from Beckman Instruments Inc.

The method was used as described (8, 9) with some slight modifications. The analyses were performed on a Nova Pak C18 column (250×4.6 mm, 4 µm) from Waters. The mobile phase was water with 0.006 M disodiumphosphate (anhydrous), adjusted with 85% orthophosphoric acid to pH 2.1. The pre-column flow rate was 1 ml/min and the post-column flow rate was 0.5 ml/min. The injected volume was 50 µl and the samples were dissolved in the mobile phase. The detector was set at 420 nm with an absorbance range of 0.1. The detection limit was 0.1 µg/g. Each analysis was performed 3×. The post-column reaction coil was a French knitted Teflon tube (11 m×1.6 mm). The reaction coil was inserted in a column heater at 99°C. The derivatization reagent, 3,5-diacetyl-1,4-dihydrolutidine, was prepared as described (9). The calibration curve was linear in the range 0.4–10 µg/g.

The formaldehyde was extracted into water to determine the formaldehyde content in emulsions. 20.00 ml dichloromethane and 20.00 ml mobile phase were added to a sample containing about 100 µg formaldehyde. After mixing, using a vibrator stirrer, the 2 phases were separated by centrifugation (3000 rpm) in 2 min. The water phase was filtered through Sep-Pak C18 from Waters conditioned with 2 ml methanol and 5 ml mobile phase, whereafter HPLC analysis was performed<sup>1</sup>. 4 different concentrations, 10, 20, 50 and 100 µg/g, of formaldehyde in cream base were prepared for calibration of the method. The recovery was 101%, 104%, 98% and 100%, respectively. The coefficient of variation (CV) was calculated to 1.9%, 1.3%, 1.7%, and 1.2%, respectively ( $n=3$ ).

#### *Comparison between the CCD method and the official method*

Diazolidinyl urea or imidazolidinyl urea in known concentrations (200–2400 µg/g) was added to 50 g Essex cream dil. Bronopol 200–2400 µg/g was added to 50 g Essex lotion. The products were kept in dark jars, 25 g in each, at room temperature for 12 months. They were then analyzed both according to the adopted method and the CCD technique.

#### *Statistical method*

The results obtained with the CCD method and the official method within EU were statistically compared using the regression line. The standard method was plotted on the  $x$ -axis while the CCD method was plotted on the  $y$ -axis. The correlation coefficient, slope and intercept were calculated for

<sup>1</sup> In the presence of formaldehyde donors the quantification of formaldehyde must be performed immediately.

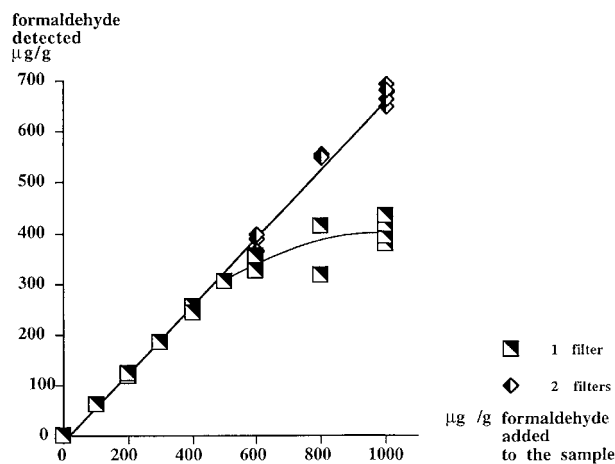


Fig. 3. Quantification of free formaldehyde from different water solutions (0.5 g) of formaldehyde. 2 filters had to be used at concentrations  $\geq 500 \mu\text{g/g}$ . A linear correlation was obtained,  $r=0.999$ , for the series of 1.0 g samples and  $r=0.995$  for the series of 0.5 g samples. (Formaldehyde detected is given as concentration in the original solution).

the regression line. The correlation coefficient was statistically evaluated and the confidence limits for the slope and the intercept were calculated using a 2-tailed  $t$ -test at a significance level of 95% (6 degrees of freedom). If the calculated confidence intervals of the slope and the intercept include 1 and 0, respectively, the regression line indicates a good agreement between the 2 analytical methods and there is no evidence for systematic differences between the methods (14).

## Results

### Formaldehyde in water

When 10 samples of 1.0 g water solutions of formaldehyde ( $50 \mu\text{g/g}$ ) were analyzed, a mean value of  $19.0 \mu\text{g}$  formaldehyde reacting on the filter was obtained. The standard deviation for the whole experimental set up was  $s=0.62$  ( $\text{CV}=3.3\%$ ).

When water solutions of formaldehyde in known concentrations from 0 to  $1000 \mu\text{g/g}$  were analyzed, a linear correlation between the concentrations in the solutions and the concentrations determined in the analyses was obtained for both the 1.0 g- sample series and the 0.5 g- sample series. An over-load effect was observed. 2 filters had to be used for samples containing  $\geq 500 \mu\text{g/g}$  (Fig. 3). 39.6% of the formaldehyde in the solution was detected when samples of 1.0 g were used, while 61.2% was detected when samples of 0.5 g were used (Fig. 4).

### Formaldehyde in cream bases

When 0.5 g samples of cream bases containing formaldehyde in known concentrations from 0 to

$400 \mu\text{g/g}$  were analyzed, a linear correlation was observed for all cream bases ( $r>0.997$ ). No formaldehyde was detected in the cream bases before addition of formaldehyde. On average, 57.7% of the formaldehyde added to Locobase had reacted on the filter. The corresponding figures were 29.6% for Essex cream, 31.2% for Essex cream dil., and 45.6% for Essex lotion. Comparing these results with those obtained for 1.0 g samples demonstrated that also for the cream bases, a higher % of formaldehyde was detected when 0.5 g samples were used (Fig. 4). Essex lotion was studied only in 0.5 g samples. No correlation between free formaldehyde detected and viscosity of the cream base was found. The figures for Locobase with the highest viscosity were comparable with those for water, while the figures for Essex cream were much lower. No difference was observed between Essex cream and Essex cream dil., although the viscosity of the diluted preparation was  $6\times$  less compared to the undiluted one (Table 1).

### Quantification of free formaldehyde from formaldehyde donors

The coefficient of variation (CV) for the total experiment was 3.2% when 10 samples of Essex cream containing diazolidinyl urea  $100 \mu\text{g/g}$  were analyzed. The corresponding figures for Essex cream dil. containing imidazolidinyl urea  $200 \mu\text{g/g}$

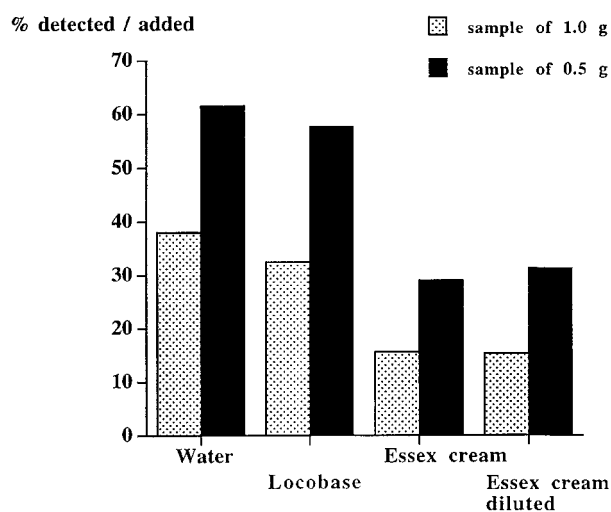
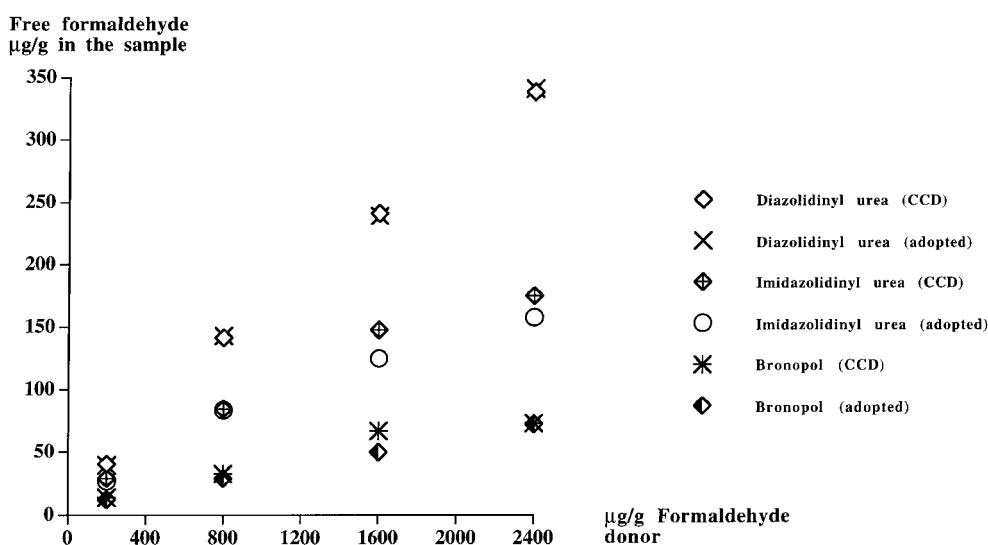


Fig. 4. Comparison between the free formaldehyde detected and the formaldehyde added to samples of 1.0 g and of 0.5 g of water and of the different cream bases. A higher percentage of free formaldehyde was detected from 0.5 g samples than from 1.0 g samples. No correlation was seen between the viscosity of the cream base and the release of formaldehyde.

Fig. 5. Quantification of free formaldehyde in cream bases preserved with the different formaldehyde donors performed with the CCD method and the official method within EU. Bronopol was added to Essex lotion, while the 2 others were added to Essex cream dil. A linear correlation between formaldehyde donor in sample and formaldehyde detected was obtained with both methods. Diazolidinyl urea: CCD method:  $r=0.996$ , adopted method:  $r=0.997$ ; imidazolidinyl urea: CCD method:  $r=0.979$  adopted method:  $r=0.982$ ; Bronopol: CCD method:  $r=0.963$ , adopted method:  $r=0.999$ .



and Essex lotion containing Bronopol 200 µg/g were 5.0% and 3.3%, respectively.

When free formaldehyde was determined for concentrations 0–3000 µg/g of diazolidinyl urea or imidazolidinyl urea in Essex cream dil. and for Bronopol in Essex lotion, a linear correlation between the formaldehyde donor in the cream base and formaldehyde detected was found in all experiments. The correlation coefficient was for diazolidinyl urea  $r=0.995$ , for imidazolidinyl urea  $r=0.994$  and for Bronopol  $r=0.983$ .

#### *Comparison between the CCD method and the official method*

The content of free formaldehyde from the formaldehyde donors was analyzed in cream bases after 1 year of storage (Fig. 5). The results obtained with the CCD technique were compared with those of the adopted method within the EU, using the regression line (Fig. 6).

Significant correlation coefficients were obtained according to the  $t$ -test, giving a linear correlation between the results from the 2 methods for all formaldehyde donors. The calculated slope and intercept do not differ significantly from 1 and 0, respectively, and there is no evidence for systematic differences between the 2 sets of results.

#### **Discussion**

A method for quantification of free formaldehyde in the presence of formaldehyde donors has been developed. The method is named the closed

container diffusion (CCD) method (Fig. 2). The method was tested on 3 different formaldehyde donors using 4 different cream bases. Our investigations show that the results obtained with this method do not differ significantly from those obtained with the official method within the European Union (EU) (8, 9) (Figs. 5, 6).

Avoidance of hidden sources of formaldehyde is essential for prevention of recurrence of allergic contact dermatitis in allergic persons. Reliable and sensitive methods for detection of formaldehyde in various products are therefore valuable tools in the patient care work. Also, for workplaces handling products preserved with formaldehyde donors, it could be of interest to check the formaldehyde content in the products. Quantification of free formaldehyde in the presence of formaldehyde donors usually gives too high and non-reproducible values with the commonly applied methods such as the "acetylacetone method" and 2,4 DNPH derivatization (8, 10), since the donors are degraded by the reagents in these methods. The HPLC method with post-column derivatization adopted within the EU is too complicated for routine work (9). The chromotropic acid method could in many cases give a semiquantitative answer that is satisfactory, but colour reactions of other volatile aldehydes and ketones disturb the results (7, 12).

Using the CCD method, quantification of formaldehyde can be performed directly on the creams without solvent extraction. Thus, it is possible for an analytical laboratory to provide 2,4-DNPH-coated filters which are used in the

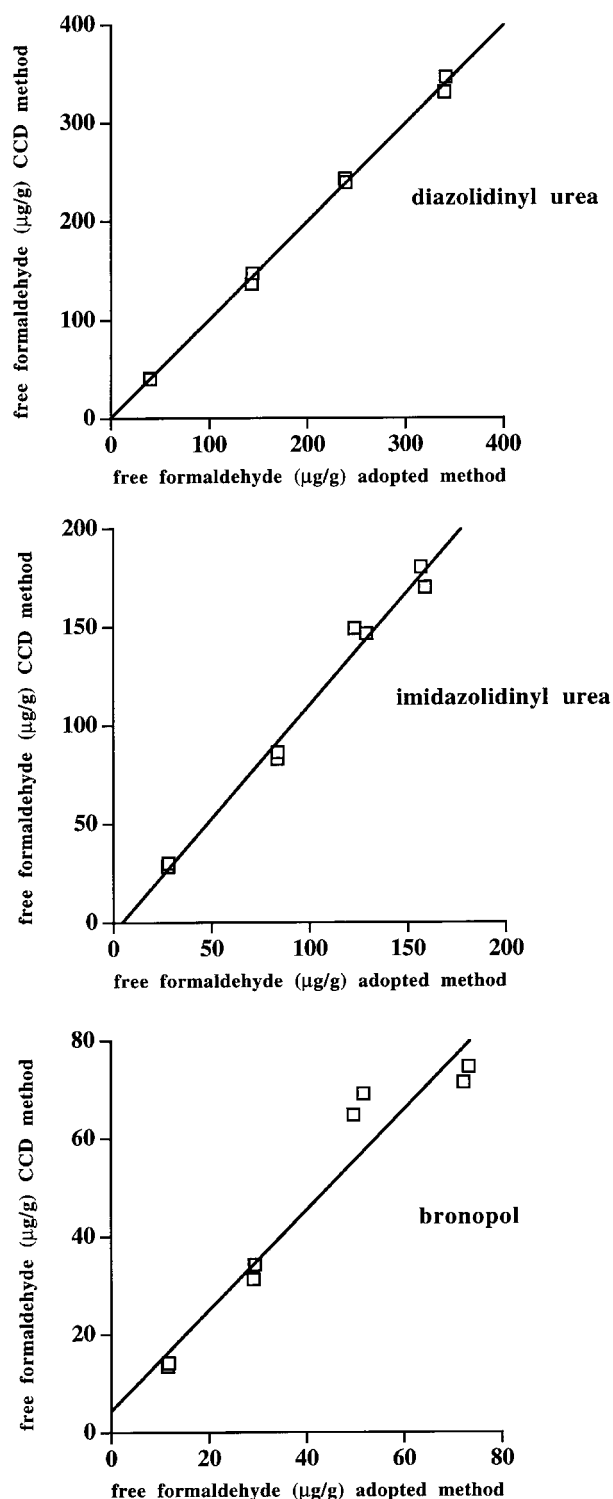


Fig. 6. Comparison of the results obtained for the 3 formaldehyde donors with the different analytical methods (Fig. 5) using the regression line. A significant linear correlation between the results from the 2 methods was observed for all formaldehyde donors. Diazolidinyl urea:  $r=0.999$ , imidazolidinyl urea:  $r=0.993$ , Bronopol:  $r=0.965$ .

experiments with the closed container and then take them back for analysis. This could be of interest for clinics and workplaces without the capacity to perform HPLC analyses on their own. However, if HPLC instrumentation is available the method used is robust and simple (10, 13).

With the CCD method, relatively higher amounts of formaldehyde were obtained on the filter with cream samples of 0.5 g than with samples of 1.0 g. The 1.0 g samples probably need a longer diffusion time before the same proportion of formaldehyde is achieved on the filter. To optimize the recovery from the product, 0.5 g samples are recommended.

It should be remembered that the CCD method does not give a recovery calculated in the same way as for the adopted method, which is based on a chemical reaction with formaldehyde in the solution. With the CCD method, it is not possible that all formaldehyde in the solution will evaporate and react on the filter. The CCD method showed good reproducibility both with water and with different cream bases. A linear correlation was obtained in all experiments between formaldehyde in the sample and the detected amount. However, when analyzed, the amount of free formaldehyde detected on the filter from the 0.5 g samples varied from 61.2% for water samples to 29.6% for samples of Essex cream. The detection limit for the method is 0.06 μg per filter, which means that the lowest detectable concentration of free formaldehyde is 0.20 μg/g in water and 0.41 μg/g in Essex cream using the CCD method. The lowest concentration described giving a positive patch test reaction in formaldehyde-sensitive individuals is 30 μg/g (15). In a more recent article (16), the threshold concentration for occluded patch test to formaldehyde in formaldehyde-sensitive patients was found to be 250 μg/g. Thus, the method is sensitive enough for analysis of patients' products and for control of labelled amounts of formaldehyde in technical products.

The variation in the diffusion of formaldehyde for the cream bases did not correlate with the viscosity of the cream bases. This was demonstrated by the fact that the same amount of formaldehyde was detected when the formaldehyde was added to Essex cream dil. as when it was added to the original cream base, although the viscosity of the diluted preparation was 6× less compared to the undiluted one. It must therefore be assumed that the difference is due to inherent properties of the cream bases. A specific standard curve should be obtained by addition of known amounts of formaldehyde to the product to be analyzed.

Two filters can be placed in the holder when high concentrations are expected or when no linear relationship is obtained between amounts in the sample and detected amounts. An over-load effect was observed for 0.5 g water solutions of formaldehyde in concentrations  $\geq 500 \mu\text{g/g}$  (Fig. 3).

Good reproducibility was obtained for the 3 formaldehyde donors investigated, both in water solutions and in cream bases. For Bronopol, the release of formaldehyde is almost zero at pH around 4 (17), which is the pH value of Essex cream, and therefore Essex lotion (pH=6) was chosen in the experiments with Bronopol. For diazolidinyl urea and imidazolidinyl urea, formaldehyde is released in measurable amounts within the range of their activity (pH 3–9) (9). It was demonstrated that more formaldehyde was released from diazolidinyl urea compared to imidazolidinyl urea, as was expected from their chemical structures (Fig. 5). Small amounts of formaldehyde were released from Bronopol, which is in accordance with the report from the producer (17).

Diazolidinyl urea is found on the list of permanently approved cosmetic preservatives allowed within the EU to be used at concentrations of 0.5% and lower (18). In the comparative experiments between the adopted method and the CCD technique, the cream bases containing known amounts of the formaldehyde donors were stored in a way that mimicked normal storage of cosmetics, i. e., 25 g of each were stored in dark closed jars at room temperature for 1 year prior to analysis. The cream base preserved with  $2400 \mu\text{g/g}$  (0.24 %) diazolidinyl urea contained  $350 \mu\text{g/g}$  free formaldehyde when analyzed (Fig. 5). If diazolidinyl urea 0.5% had been used in the actual experiment, the content of free formaldehyde would have exceeded  $500 \mu\text{g/g}$ , since we have demonstrated a linear correlation between the content of diazolidinyl urea in the cream base and the amount of formaldehyde detected.  $500 \mu\text{g/g}$  is the labelling limit for content of formaldehyde in cosmetic products within the EU (19, 20).

The CCD method will be further investigated using other formaldehyde donors with a different reactivity and another usage.

### Conclusion

The closed container diffusion (CCD) method for quantification of free formaldehyde in the presence of formaldehyde donors was found to be useful and reliable for quantification of free formaldehyde from the formaldehyde donors diazolidinyl urea, imidazolidinyl urea and Bronopol in water and in 4 different cream bases. Our investigations show that the results obtained with this method do

not differ significantly from those obtained with the official method within the EU. The method is sensitive enough for analysis of patients' products and for control of labelled amounts of formaldehyde in technical products without solvent extraction.

The variation in the diffusion of formaldehyde for the cream bases did not correlate with the viscosity of the cream bases. Therefore, a specific standard curve should be obtained by addition of known amounts of formaldehyde to the product to be analyzed.

Furthermore, as a result of our studies, we observed a risk of exceeding the labelling limit for free formaldehyde in cosmetic products when using the highest amount of diazolidinyl urea allowed.

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