TECHNICAL NOTE

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The Ninhydrin Process in Supercritical Carbon Dioxide

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ABSTRACT: Ninhydrin is a widely used reagent for the development of latent fingerprints on porous surfaces. For the past 20 years 1,1,2-trichlorotrifluoroethane (CFC113) has been used as the solvent of choice in the UK for ninhydrin because of its non-toxicity and non-flammability. With the phasing out of CFC solvents under the terms of the Montreal Protocol on the Control of Ozone Depleting Substances, the need for a suitable replacement has arisen. Supercritical CO_2 has been found to be a potential replacement for CFC113 in the ninhydrin process. Furthermore, the present processes are two-stage (impregnation of reagent followed by development in an oven), whereas using supercritical CO_2 development of fingerprints can be carried out in one stage.

KEYWORDS: forensic science, ninhydrin, fingerprints, latent fingerprints, supercritical carbon dioxide, carbon dioxide

Ninhydrin is the most commonly used reagent for developing latent fingerprints on paper and other porous surfaces. 1,8-Diazafluorene-9-one (DFO) is being used increasingly in sequence with ninhydrin on exhibits from major crime. Ninhydrin and DFO react with amino acids present in eccrine sweat to give purple or fluorescent fingerprints respectively (1,2). The ninhydrin and DFO formulations currently used by British Police Forces employ 1,1,2-trichlorotrifluoroethane (CFC113) as the main carrier solvent (3). Prior to the introduction of the use of CFC113 in the UK for the ninhydrin process, many highly flammable solvents were used including acetone (4) and petroleum ether (5). CFC113 was introduced specifically to reduce the explosion and fire hazards associated with the use of highly flammable solvents. Paper evidence is currently treated by passing the paper through a shallow trough of the reagent solution and allowing the surface to dry completely in the open atmosphere. The solution is also brushed onto cardboard articles and wall paper at scenes of crime. The latent fingerprints are then developed by heating the paper in a specially adapted humidity oven at 80°C and 65% relative humidity. DFO treated surfaces, however, are treated at 100°C with no added humidity (6).

CFC113 has several properties that makes it ideal as a solvent in the ninhydrin and DFO processes. CFC113 is: (i) non-flammable; (ii) non-toxic; (iii) volatile; (iv) does not cause diffusion of handwriting. However as CFC113 is an ozone-depleting substance, industrial production of the solvent for purposes other than those granted essential user status ceased within the European Community at the end of December 1994. The use of CFC113 in fingerprinting had been granted essential user status until the end of 1995. A safe, cost effective replacement is therefore urgently required.

As a result many countries have introduced a range of ninhydrin formulations that use hydrocarbons as the main carrier solvents. These solutions are all flammable hence their use introduces undesirable safety hazards. Hydrochlorofluorocarbons (HCFCs) are potential non-flammable CFC replacements but represent only an interim solution as these compounds are due to be phased out by the year 2015.

Supercritical fluids have been studied by numerous researchers wishing to replace organic solvents used in industrial processes (7,8). As supercritical CO_2 is non-flammable, volatile and non-hazardous in low concentrations, a feasibility study into its use as a replacement for CFC113 in the ninhydrin process has been undertaken in collaboration with Express,[3] a division of the University of Leeds Innovations Ltd.

A supercritical fluid is formed when a gas is maintained above its critical pressure p_c and temperature T_c. Supercritical fluids possess intriguing physical properties intermediate between gases and liquids: they exhibit the diffusion rates typical of gases, but can dissolve organic compounds like conventional liquid solvents. The fast diffusion rates should enable a reagent dissolved in a supercritical fluid to be deposited significantly further and faster through a porous solid than a conventional reagent solution, hopefully enabling closely packed paper articles to be treated simultaneously.

Preliminary Experiments

For supercritical CO_2 to be suitable as a CFC113 replacement, it must be able to dissolve ninhydrin but not amino acids such as glycine and serine present in the latent fingerprint. A simple experiment to measure the relative solubilities of ninhydrin, DFO, serine and glycine in supercritical CO_2 using column chromatography was therefore performed. The results of this study were very encouraging. Neither of the two amino acids were eluted from the supercritical CO_2 column under the conditions used (50°C, 300 bar). Ninhydrin however was eluted from the column in approx. 1.5 min. DFO could not be eluted in pure supercritical CO_2, but

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could be dissolved by the addition of 5% methanol to the supercritical CO_2 with an eventual elution time of 3.75 min.

Prototype Reactor

To further assess the viability of using supercritical CO_2 as a replacement for CFC113 in the ninhydrin process, a small prototype reactor was constructed, as shown in Fig. 1. The cell or treatment vessel has a capacity of 165 cm^3 and is equipped with an electrical heater surrounded by a ceramic insulator. CO_2 is introduced into the vessel from a standard F size cylinder using a high pressure pump, the pressure inside the reactor being constantly measured using a pressure gauge. Convection of the supercritical CO_2 around the vessel is accomplished using an external unheated pipe that connects the top and bottom of the reactor together. At the end of the treatment period, the pressure is released by opening a valve to the atmosphere.

Experiments on Fingerprint Development

The reactor was used to perform two sets of experiments. The first set of experiments compared the abilities of a standard CFC113 ninhydrin formulation and ninhydrin in supercritical CO_2 to develop planted fingerprints on white photocopying paper. The second set of experiments examined whether fingerprints could be developed on used checks.

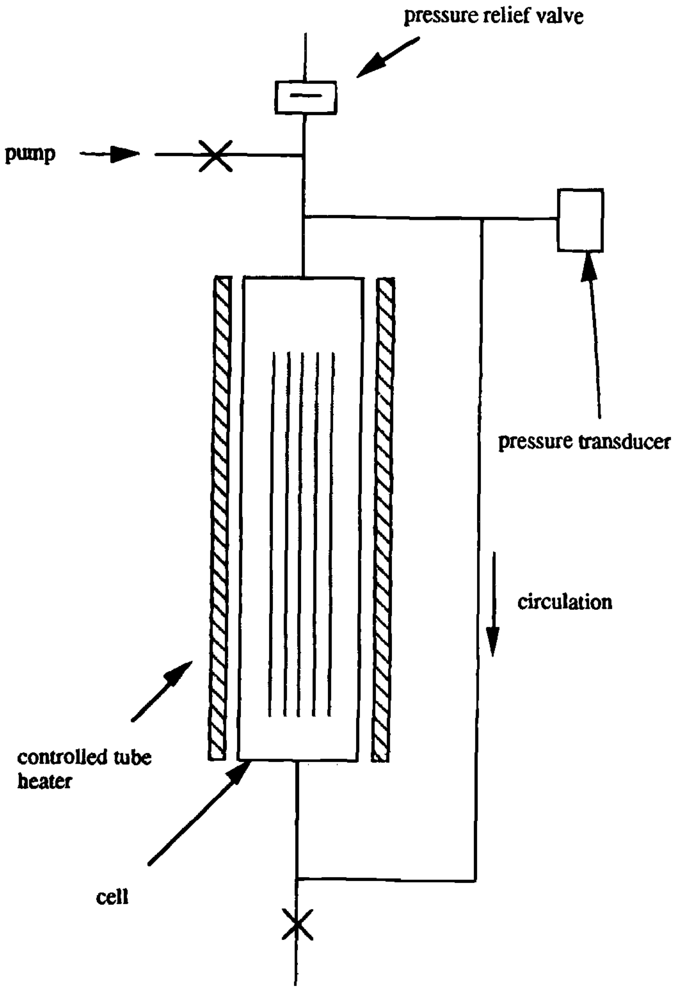

FIG. 1—*Schematic diagram of prototype reactor for fingerprint detection.*

Development of Deposited Fingerprints

Deposited fingerprints (10 depletions) were taken from each of ten donors. The fingerprints were split, one half treated in the prototype supercritical CO_2 reactor, the other half treated with the CFC113 ninhydrin formulation. Three sets of conditions were examined (Table 1). All the experiments were carried out at 80°C. The amounts of acetic acid and water were roughly controlled by placing two pieces of Whatman filter paper, one soaked in acetic acid and the other in water, at the bottom of the cell. In experiment 2, two 2 cm × 2 cm pieces of Whatman paper, one soaked in acetic acid and the other soaked in water, were used. In experiment 3, two 9 cm diameter discs of Whatman paper were used, again one soaked in acetic acid and the other in water.

There was no fingerprint development under the conditions used in Experiment 1. Very little ninhydrin was observed to dissolve despite maintaining the pressure between 235 and 300 bar. The conditions used in Experiment 2 however resulted in good fingerprint development, as shown in Fig. 2*a*. This result could be due to the solubilizing effects of acetic acid and water on ninhydrin, together with an increase in the rate of production of Ruhemann's purple. It has been known for many years that the addition of acetic acid to formulations of ninhydrin result in the accelerated development of fingerprints, especially when the treated articles are heated in humid environments. In Experiment 3, the planted split fingerprints were pinned closely together and treated to see if the increased diffusion rates of supercritical fluids would enable the simultaneous development of all of the fingerprints. Again fingerprints were developed but in this case some ridge diffusion also occurred, as shown in Fig. 2*b*. This could be due either to the presence of too much water or because of an excessively long reaction time.

Development of Fingerprints on Fraudulently Passed Checks

An experiment to develop latent fingerprints on fraudulently passed checks was also undertaken. Six checks were treated simultaneously in the CO_2 reactor containing 0.5 g ninhydrin. The reactor was pressurized up to 300 bar and heated to 80°C for 1 h maintaining the pressure between 250 and 300 bar. After 1 h the pressure was released over 15 min. During the reaction time 0.02 g of the ninhydrin had been dissolved.

Good fingerprint development occurred on several of the checks. It was originally hoped that the fast diffusion rates of supercritical fluids compared to conventional solvents would increase the rate of ninhydrin distribution through the paper thereby enabling the treatment of closely packed paper. Unfortunately some of the checks showed signs of limited ninhydrin diffusion. This is most dramatically seen on the edges of some of the developed fingerprints where ridge detail suddenly disappears, as shown in Fig.

TABLE 1—*Experiments on the development of deposited fingerprints.*

Experiment	Wt. of ninhydrin (g)	Pressure (bar)	Time (min)	Chemical Additives
1	0.8	300–235	30	None
2	0.1	300–250	30	Acetic acid Water*
3	1.0	300–280	60	Acetic acid Water*

*The quantities of acetic acid and water were poorly controlled.

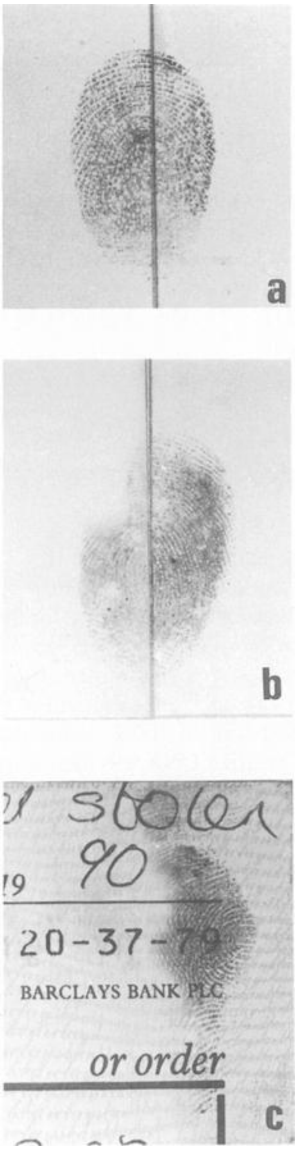

FIG. 2—*Fingerprints developed from ninhydrin and acetic acid in supercritical CO_2 at 80°C:* (a) *deposited fingerprints with the right hand side developed conventionally and the left-hand side developed in CO_2;* (b) *same experiment but with nine samples pinned closely together, showing signs of ridge diffusion;* (c) *fraudulently passed check.*

2*c*. There was no indication of diffusion of handwriting or printing inks on any of the checks treated.

Conclusions

The described experiments demonstrate that supercritical CO_2 can be used as a solvent for ninhydrin in the development of latent fingerprints in a one-stage process. The process is dependent on the presence of water and acetic acid, but the addition of excessive quantities of these compounds does cause significant ridge diffusion.

It should also be possible to apply DFO to paper evidence using the technology outlined in this paper, but it must be noted that supercritical CO_2 does not constitute a "drop-in" replacement for CFC113. The size of the apparatus needed to use supercritical CO_2 as a solvent will limit the size of article that can be treated: treating large cardboard boxes, for example, is simply not feasible using existing supercritical CO_2 vessels.

This technology probably shows most promise for more advanced forensic techniques, possibly involving the extraction and analysis of trace samples of illicit materials from paper evidence in supercritical media. It may also be possible to use the technique for the application of other types of fingerprint reagent.

References

(1) Lee HC, Gaensslen RE, editors. Advances in fingerprint technology, Elsevier, New York, 1991.
(2) Kent T, editor, Manual of fingerprint development techniques, British Home Office, London, 1992 and references herein.
(3) Morris JR, Goode GC, NFN—an improved ninhydrin reagent for detection of latent fingerprints. Police Res Bull 1974;24:45–53.
(4) Oden S. Detection of fingerprints by the ninhydrin reaction. Nature 1954;173(4401):449–50.
(5) Crown DA. The development of latent fingerprints with ninhydrin. J Crim Law Criminol Police Sci 1969;60(2):258.
(6) Hardwick S, Kent T, Sears V, Winfield P. Improvements to the formulation of DFO and the effects of heat on the reaction with latent fingerprints. Fingerprint World 1993;19(73):65–69.
(7) Clifford AA, Bartle KD. Chemistry goes supercritical. Chem Br 1993;29(6):499–502.
(8) McHugh MA, Krukonis VJ. Supercritical fluid extraction. Butterworth-Heinemann, Boston, 1994.

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