Int J Legal Med (1997) 110 : 55–62 

ORIGINAL ARTICLE

**L. Pötsch · G. Skopp · G. Rippin**

# A comparison of $^{3}$H-cocaine binding on melanin granules and human hair in vitro

Received: 21 August 1996 / Received in revised form: 4 December 1996

**Abstract** The in vitro experiments on the interaction of $^{3}$H-cocaine and melanin from *Sepia officinalis* confirmed the existence of drug binding sites on melanin granules. The results suggested that the binding of $^{3}$H-cocaine to melanin could be analyzed by assuming that the binding to the surface of pigment granules is analogous to the adsorption of a drug on a solid and follows Langmuir adsorption isotherm type I. Scatchard analysis indicated heterogeneity of binding sites. Structural and chemical alterations caused by isolation of the melanoproteins, which are heterogenous in nature and show different physicochemical properties, are considered to be most crucial. The studies on hair samples confirmed that melanin-drug interactions occur on the surface of melanin granules. These seem to be of minor importance compared to the drug-melanoprotein loading during melanogenesis for the observed influence of pigmentation on the drug content of hair fibers. From the results it was concluded that in vitro studies on melanin provide limited information and even drug-soaked hair must be regarded as inappropriate for the study of melanin-drug-binding in hair.

**Key words** Hair analysis · Drug binding · Melanin granules · Cocaine · Drug-melanin-complex

## Introduction

Toxicological analysis of hair is becoming a popular method for investigating previous, chronic use of illicit drugs. Various analytical methods such as radioimmunoassay, chromatography and mass spectrometry have been reported [6, 11, 25–27, 33, 56, 64]. Although the number of reports on substances detected in hair has increased tremendously in the last years, the mechanisms involved in the incorporation and the deposition of drug molecules are still unknown in detail and forensic interpretation of the results obtained from hair samples remains crucial. Only a few attempts have been made to determine the origin, the stability and the fate of drug molecules in hair and to elucidate the large numbers of practically unanswered questions in hair analysis [9, 16, 24, 35–37, 43–46, 55, 59, 60]. According to the biochemical concept of endogenous drug incorporation, the hair lipids (cell membrane complex), the hair proteins (keratin intermediate filaments; intermediate filament associated proteins) and melanin (melanin granules) may be considered to be the main sources for endogenously entrapped drug molecules in keratinized hair fibers [46].

Many investigators have demonstrated the affinity of natural and synthetic melanins for various drugs by in vivo and in vitro studies [1, 3, 5, 30, 31, 32, 39, 49, 50, 58, 62, 63]. It is generally accepted that the ability of melanin-containing tissue to accumulate and retain these drugs is remarkable [18]. From preliminary observations it was concluded that hair is not an exception [41]. The results of a few studies on both human and animal hair fibers have already indicated that the interaction of drugs with the melanin granules may have implications in assaying clipped scalp hair [8, 11, 14, 37, 47, 48, 53, 55, 65]. Nakahara et al. [37] classified 20 drugs of abuse for their incorporation into hair. The drug-melanin affinity of the particular substance was calculated from in vitro studies with synthetic melanin. The ranking was in accordance with observations in hair analysis showing a high melanin affinity for cocaine. Reid et al. [53] presented another model to estimate the influence of hair pigmentation on the drug content in hair. They soaked hair fibers of different colors in acetate buffer containing 2 μg benzoylecgonine/mL at elevated temperatures for 42 h and observed differential drug absorption in the order black > brown > blond hair fibers.

L. Pötsch (✉)
Institut für Rechtsmedizin, Johannes Gutenberg Universität,
Am Pulverturm 3, D-55131 Mainz, Germany

G. Skopp
Institut für Rechtsmedizin, Ruprecht-Karls-Universität,
Vossstrasse 2, D-69115 Heidelberg, Germany

G. Rippin
Institut für Medizinische Statistik und Dokumentation,
Johannes Gutenberg Universität, Langenbeckstrasse 1,
D-55131 Mainz, Germany

A specific aim of the present experiments was to compare and contrast frequent in vitro designs in order to evaluate and to discuss their use and relevance for studies on drug-melanin binding in hair.

## Materials and methods

### Materials

$^{3}$H-cocaine, levo-(–)-[benzoyl-3, 4-$^{3}$H(N)] (specific activity: 1054.5 GBq mmol$^{-1}$) was obtained from DuPont NEN (Bad Homburg, Germany). Melanin (*Sepia officinalis*), tetraethylammonium hydroxide (20% w/w, aqueous solution), dithioerythritol, papain, Dulbecco's phosphate buffer pH 7.4 (PBS) were obtained from Sigma (München, Germany). Other chemicals were of analytical grade and were purchased from Merck (Darmstadt, Germany). The liquid scintillation cocktail (rotiszint) was from Roth (Karlsruhe, Germany). Centricon separation equipment was supplied by Amicon (Witten, Germany).

Natural hair of black color was obtained from a drug-free, female Asian. Samples of natural hair of brown, red and blond color were collected from healthy drug-free Caucasian volunteers (children aged 8–12 years). The hair strands were clipped close to the scalp.The hair samples were rinsed under tap water for 3 min, blotted between filter papers, air dried and stored in the dark at room temperature until use.The proximal 3 cm of the hair fibers was used for the experiments.

### Methods

The ligand concentration ($^{3}$H-cocaine) was estimated by mixing 100 µL of the particular specimen with 4 mL scintillation fluid for subsequent quantitation by liquid scintillation spectrometry (LSS) in a Liquid Scintillation Analyzer Tricarb 1600 TR (Canberra Packard, Frankfurt, Germany). The tests on *Sepia* melanin were run 4 or 6 times unless otherwise specified. The experiments on human hair powder, human hair fibers and on melanin granules isolated from human hair were performed in duplicate. Controls such as blank buffer solutions, melanin suspensions without drug, and buffer solutions with 140 pmol/mL $^{3}$H-cocaine were always included. The portion of analyte bound to the matrix under investigation was calculated from the difference of the labelled substance added and the concentration of the radiolabelled drug in the supernatant at given times. Incubation was performed at 37° C. The mean of the assay runs (range: 5%) was used for data analysis.

To analyze the binding characteristics of $^{3}$H-cocaine on *Sepia* melanin a program for general non-linear regression (NLIN procedure in the SAS system, SAS Institute US, Cary, NC) was used. Electron microscopical investigations of *Sepia* melanin, pulverized hair samples and of melanin granules isolated from hair were performed in a Zeiss 962 scanning microscope at 10 KV (Zeiss, Oberkochem, Germany).

Hair samples were pulverized in a ball mill (Retsch, Haan, Germany). Isolation of melanin granules from hair of different colors was performed from 200 mg hair portions cut into snippets. Three different protocols for isolation were used. Method 1: the hair sample was stirred at 85° C in 5 M NaOH for 8 h. Method 2: the hair sample was dissolved in 7.5 ml of tetraethylammonium hydroxide at room temperature overnight. Method 3: the hair sample was incubated in 10 ml of the hair digest solution described by Baumgartner [2] at 37° C for 5 days. After hair dissolution (methods 1–3) the melanin granules were separated by centrifugation followed by five repeated washings in 50 mL PBS.

### Experimental designs

In vitro studies on *Sepia* melanin

Melanin granules isolated from cuttle fish (*Sepia officinalis*) are a proposed standard for natural melanins [4, 10]. Like all the synthetic melanins obtained either by biosynthetic oxidation of L-dopa or L-tyrosine or from chemical oxidation of L-tyrosine, this natural pigment represents pure eumelanins. However, in contrast to synthetic melanins, the eumelanins in *Sepia* melanin are bound to a proteinaceous backbone, being melanoproteinaceous biopolymers such as the pigment in melanin granules present in hair. Although little is known about the nature or structure of the protein component the chromophoric component is bound to and which is reported to account for 6–50% of the weight, *Sepia* melanin was considered a suitable model to study drug-melanin complex formation on the surface of the melanin granules.

*Scanning electron microscopy (SEM)*

A *Sepia* melanin monolayer preparation was sprayed with a layer of gold and viewed in a scanning electron microscope at 10 KV to evaluate particle size distribution and surface morphology of the *Sepia* melanin batch used for the following experiments. Additionally isolated melanin granule preparations from hair were screened for purity and morphology. Pulverized hair was also investigated by SEM to determine its homogeneity and characteristic morphological particles.

*Melanin binding as a function of incubation time*

Drug binding to melanin was measured by incubating constant amounts of $^{3}$H-cocaine (140 pmol/mL) with melanin suspensions containing 0.5 mg, 1.0 mg and 2.5 mg melanin/mL in sodium phosphate buffer. Melanin granules were sonicated in phosphate buffer for 10 min to prevent aggregate formation. During sonication, aliquots of 1 mL were taken to guaranty homogenously dispersed samples. $^{3}$H-cocaine was added to each vial which was subsequently incubated at 37° C. At given times (3 min, 5 min, 10 min, 30 min, 60 min, 120 min, 180 min) 200 µL was removed, centrifuged at 6000 rpm for 1 min, and 100 µL of the supernatant was quantified by LSS. The concentration of bound cocaine was taken as the difference between the amount initially added and the amount remaining in the supernatant.

*Melanin binding as a function of drug concentration*

A constant weight of melanin granules (1 mg) suspended in 1 ml PBS was incubated for 60 min at 37° C with varying concentrations of $^{3}$H-cocaine (140 pmol/mL; 280 pmol/

mL; 560 pmol/mL; 35 nmol/mL; 70 nmol/mL, 350 nmol/mL) and 100 µL were removed for counting and calculating the concentration.

*Effect of ethanol in the binding assay*

Because the radiolabelled stock solution of [3]H-cocaine was in ethanol, the influence of ethanol on melanin binding properties was investigated. Assays with 1 mg melanin granules and a concentration of 140 pmol/mL [3]H-cocaine were run in three series at 37° C for 60 min. The volume percentages of ethanol in the binding experiments were 10%, 20% and 50%.

In vitro tests on human hair

*[3]H-cocaine uptake by pulverized samples from different hair colors*

Aliquots of 20–40 mg of hair powder from different colored hair was incubated with 140 pmol of [3]H-cocaine in either 1 mL PBS pH 7.4, 1 mL acetate buffer pH 4.0 or in 1 mL hair digest solution pH 9.1 as described by Baumgartner [2] at 37° C for 60 min. The amounts of drug adsorbed/absorbed by the hair powders were assayed as described above.

[3]H-cocaine uptake by hair fibers of different colors

Loops of about 50 hair fibers of different color were placed upside down in reaction tubes containing 140 pmol [3]H-cocaine in PBS. Both ends of the hair loops were outside the fluid. After incubation for 3 days at 37° C the hair fibers were removed, carefully blotted between filter paper, immersed in 10 mL methanol at 4° C for 5 min and air dried. The middle segments (2 cm in length) were cut and used for further investigations. The end segments of the hair loops were discarded. From each center-piece 12 hair fibers (0.8–1.2 mg) were dissolved in 200 µL tetraethylammonium hydroxide at 60° C for 2 h. Prior to LSS 4 ml of rotiszint was added.

[3]H-cocaine binding to melanin granules isolated from different colored hair

After the last wash the melanin pellets were resuspended in 1 ml PBS containing 140 pmol [3]H-cocaine. After 1 h at 37° C the vials were briefly centrifuged and 100 µL of the supernatant was analyzed by LSS.

## Results

In vitro studies on *Sepia* melanin

SEM investigations (Fig. 1) showed that the particle size distribution in the *Sepia* melanin charge investigated was quiet broad. The size of *Sepia* melanin granules varied from 0.4–26 µm. The size of melanin granules isolated from human hair was in the range of 0.3–1 µm. In the hair powder samples fragments < 50 µm were present. In all monolayer preparations particles could be identified as

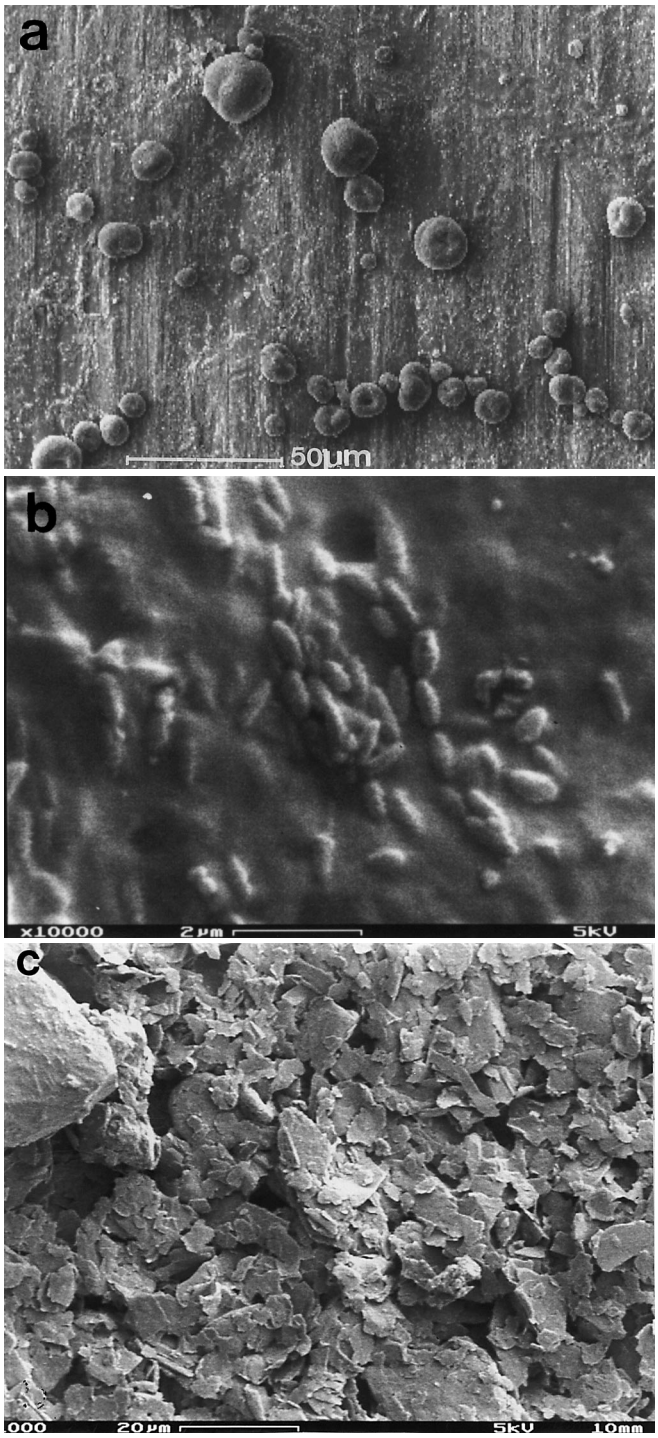


**Fig. 1** SEM investigations. **a**: *Sepia* melanin showing broad particle size distribution, 10 KV/10 mm, magnification 500 ×. **b**: melanin granules isolated from human hair by 5 M NaOH, 5KV/10 mm, magnification 10000 ×. **c**: bulk preparation of pulverized Asian hair, 5 KV/10 mm, magnification: 1000 ×

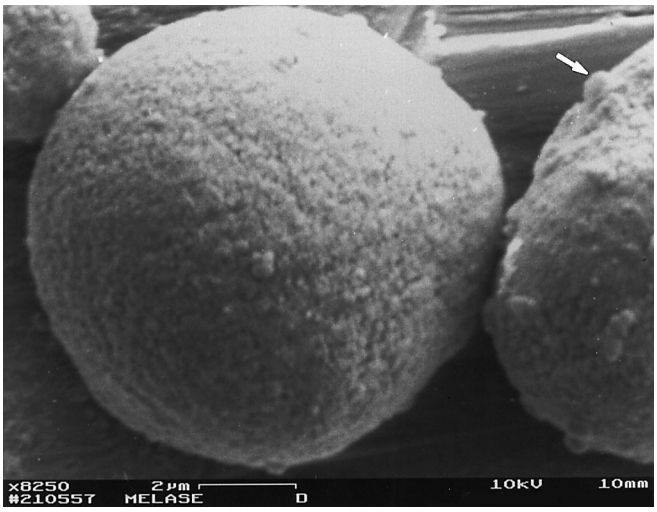


**Fig. 2** SEM investigation of *Sepia* melanin. Granular surface morphology. Accumulation of smaller melanin granules onto the surface, 10 KV/10 mm, magnification 8250 ×

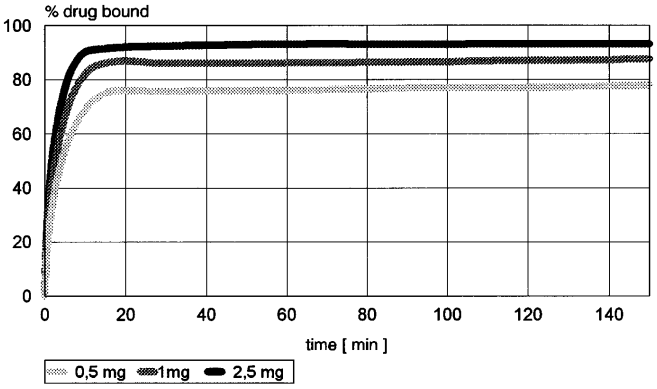


**Fig. 3** Time course of $^3$H-cocaine accumulation on *Sepia* melanin. Amount of melanin granules offered to 140 pmol $^3$H-cocaine in 1 ml PBS pH 7.4: 0.5 mg; 1 mg; 2.5 mg

fragments of cuticles and cortex cells. Only very few liberated melanin granules could be found, some of them showing severe mechanical damage (data not shown). Aggregation of small pigment granules onto the surface of larger ones was sometimes present in *Sepia* melanin (Fig. 2).

The effect of incubation time on the amount of $^3$H-cocaine bound to melanin is shown in Fig. 3. It can be seen that the time taken to reach equilibrium for melanin-drug binding is rather short and most of the binding occurs rapidly within a few minutes after incubation. When the amount of melanin offered to the drug was increased (0.5 mg; 1 mg; 2.5 mg), the rates for the formation of drug-melanin complexes also increased.

In the experiments using a constant concentration of labelled cocaine (140 pmol) with varying amounts of melanin granules added, a plot of the reciprocal of the experimentally measured amount bound per milligram of melanin as a function of the reciprocal of the free cocaine concentration was linear (Fig. 4). The results obtained sug-

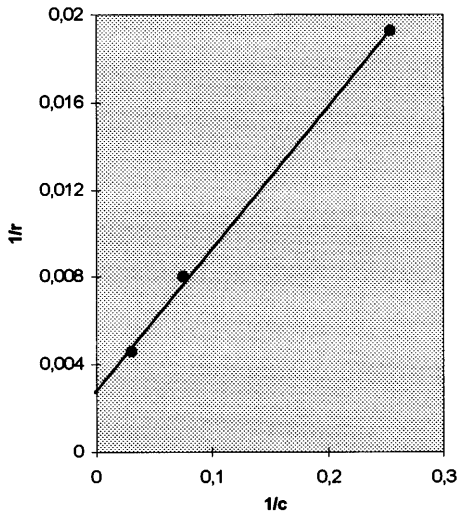


**Fig. 4** Langmuir plot; r: reciprocal of amount of drug bound to *Sepia* melanin (1 mg/mL) versus c: reciprocal of molar free drug concentration of $^3$H-cocaine. Initial drug concentration: 140 pmol $^3$H-cocaine/mL

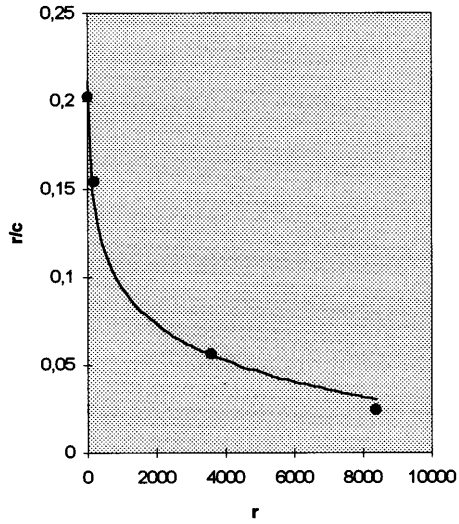


**Fig. 5** Scatchard plot for the melanin-binding of $^3$H-cocaine on *Sepia* melanin. r: pmoL of $^3$H-cocaine bound per mg melanin. c: concentration of the free drug

gested that the binding of $^3$H-cocaine to *Sepia* melanin could be analyzed by assuming that the binding is analogous to the adsorption of a drug on a solid and follows Langmuir adsorption isotherm type I [28]. The constant K, which is related to the affinity or strength of the interaction between $^3$H-cocaine and melanin under the experimental conditions, was determined as $K = 63 \times 10^9\ M^{-1}$.

When cocaine binding to melanin was studied using a constant amount of melanin granules (1 mg) and increasing amounts of cocaine were added (140 pmol–350 nmol) Scatchard plot analysis was curvilinear (Fig. 5). It was assumed that the observed nonlinearity indicated heterogeneity of binding sites on the surface of melanin granules.

**Table 1** Summary of the in vitro binding studies of $^{3}$H-cocaine (140 pmol/mL) in PBS pH 7.4 on *Sepia* melanin and human hair samples

| Matrix | Sepia melanin | Human hair powder | Human hair fibers | Melanin granules isolated from human hair |
|---|---|---|---|---|
| Type of melanin | Eumelanin | Eumelanin Pheomelanin | Eumelanin Pheomelanin | Eumelanin Pheomelanin |
| Time of drug exposure | 1 hour | 1 hour | 3 days | 1 hour |
| Percentage of the offered drug adsorbed/absorbed | 87%/mg Langmuir adsorption | 0.16–0.66%/ mg hair | 3.6–7.2%/ mg hair | 0.16–0.41%/ mg hair |
| For | | Mean value | Mean value | Mean value |
| black | 121.8 pmoL/mg | 0.87 pmoL/mg | 10.1 pmoL/mg | 0.50 pmoL/mg |
| brown | | 0.86 pmoL/mg | 6.6 pmoL/mg | 0.40 pmoL/mg |
| red | | 0.83 pmoL/mg | 5.2 pmoL/mg | 0.35 pmoL/mg |
| blond | | 0.83 pmoL/mg hair | 5.0 pmoL/mg hair | 0.32 pmoL/mg hair |

The presence of ethanol in the incubation medium had a strong effect on the melanin-drug-complex formation. With increasing concentrations of ethanol a decrease in the melanin-cocaine interaction (from 87% in the absence of ethanol down to 29% in the presence of 50% ethanol) was observed (data not shown).

In vitro tests on human hair

The binding assays run for 1 h performed in PBS pH 7.4, acetate buffer pH 4.0 or in hair digest solution pH 9.0 revealed no significant differences in $^{3}$H-cocaine binding on hair powder from different colored hair (Table1). All hair fiber samples took up $^{3}$H-cocaine (Table 1), however, the amount was less than 10% of the drug concentration in the starting solution. A graduation depending on the color of hair was observed. Asian black hair (10.1 pmol/mg) absorbed more than Caucasian brown (6.6 pmol/mg), red (5.2 pmol/mg) or blond (5.0 pmol/mg) fibers.

All melanin granule preparations from human hair showed drug-melanin accumulation. For melanin granules isolated in 5 M NaOH the binding was slightly lower. A tendency of drug binding to melanin granules was observed in the order black > brown > red ≈ blond hair color. However, highly significant differences could not be established under the present experimental conditions. The range of $^{3}$H-cocaine accumulation on melanin granules isolated from black hair was 0.42–0.52 pmol/mg hair, from brown hair 0.28–0.52 pmol/mg, from red hair 0.26–0.48 pmoL/mg and from blond hair 0.22–0.42 pmol/mg hair, dependent on the isolation method.

## Discussion

*Sepia* melanin is suggested to be a suitable model to study principles of drug-melanin-complex formation on the surface of melanin granules. The nature of the drug-melanin interactions is not exactly known. It is well established that melanin polymers contain a large number of free carboxylic acid residues which are responsible for the cation exchange properties observed for melanins and thought to provide most of the ionic binding sites. The amino acid residues detected in melanoproteins possess acidic, basic, hydroxyl, and sulfhydryl group side chains [68]. Non-electrostatic binding mechanism such as van der Waals forces, hydrophobic forces or charge transfer complexes are probably also involved [1, 3, 29, 50, 58, 63]. Tjälve et al. suggested that for each drug even several classes of binding sites may be implicated [62, 63]. Indeed, Scatchard analysis of the binding data [57] indicated heterogeneity of binding sites for cocaine in the present study. The results obtained on *Sepia* melanin were similar to the binding characteristics reported on cocaine binding to synthetic melanins [3, 39, 58].

The biochemical concept of endogenous drug incorporation during hair fiber formation, which can explain phenomena currently observed in hair analysis, suggests that the melanin granules are one of the sources for drug molecules in keratinized hair fibers [46]. Briefly, in a melanocyte a particular drug substance may be bound to the polymer or to the melanoprotein during melanogenesis according to its melanin affinity and become entrapped during melanin granule formation. This mechanism was already reported for $^{14}$C-nicotine incorporation [30]. In addition, melanin accumulation may occur on the surface of the pigment granules in the melanocytes as well as inside the matrix cells of the hair follicle. This accumulation of drug molecules on the surface of the melanin granules could be studied, whereas melanin-drug interactions that may occur during melanogenesis cannot be simulated in vitro. The results (Table 1) indicated that drug-melanin interaction on the surface of melanin granules does occur, but must be regarded to be of minor importance compared to the drug-melanin interactions in melanin granule formation during melanogenesis. The amount of drug that accumulated on the surface of the melanin granules in the present in vitro experiments cannot explain the observed significant differences of the drug concentration for vari-

ous substances in pigmented hair compared to non-pigmented hair fibers in vivo.

In the hair powder experiments the incubation time was obviously too short for the drug to gain access to the binding sites inside the hair fragments, which still encase the melanin granules (Fig. 1).The results of the in vitro experiments in the present study clearly demonstrated that drug-soaked hair powder or drug-soaked hair fibers are rather inappropriate materials to study melanin-drug binding in hair. A higher drug content showing a tendency in order of magnitude black > brown > red ≈ blond was found in hair fibers, that had been soaked for 3 days. These findings are in accordance with the results reported by Reid et al. [53]. However, the portion of drug molecules bound to melanin granules could not be separated from the drug portion bound to other components in the keratinized fibers. As for all natural tissue-bound biopolymers the problem of alteration by isolation is present. The inherent stability of a biopolymer towards heat, light, acids, bases and even water results in chemical and structural alterations, which cannot be excluded for isolated melanin granules [13, 19] and potentially affect in vitro binding studies. The most crucial factor seemed to be the different solubility of pheomelanins and eumelanins and the alterations of the proteinaceous backbone component to which the chromophore is bound [10, 12, 15, 21, 34, 51, 52, 61]. Most of the previous studies on natural melanins were performed on those fractions obtained by strong acid or alkali treatment from pigmented tissues. It should be born in mind, that these melanins originate mainly from the surface of the pigment granules and neither non-oxidative alkaline nor acidic conditions can completely dissolve the melanin granules.

All of the isolation procedures for melanin granules from hair resulted in colored supernatants indicating that melanins had been liberated. This portion of melanins, the amount of drug substances associated with this fraction as well as drug desorption from the granule surface during sample preparation procedures is suggested to be the main source for the observed difference in drug concentrations, which was attributed to result from differences in hair pigmentation. Therefore exact determinations of the amount of a drug bound to the surface of melanin granules in hair seems questionable, and in the present study the melanin granule pellets were not separated after the soaked hair fibers had been dissolved. Green and Wilson [14] as well as Wilson et al. [67] estimated the portion of melanin dissolved by 1 M NaOH from rat and human hair by UV-spectroscopy. Indeed, they observed a certain correlation of the liberated melanin and the methadone concentration in the black colored fibers. However, this procedure might poorly be transferred to human hair, for neither the ratio of pheomelanin to eumelanin content in the solution can be calculated by UV-spectroscopy nor does the amount of liberated melanins reflect the melanin content of the entire melanin granules and thus the melanin content of the particular hair sample.

From animal studies it was concluded that eumelanins rather than pheomelanins may be a decisive factor influencing the drug content of a hair fiber and a kind of dose-dependent influence of pigmentation was observed [47, 48]. From the biology of hair pigmentation it can be predicted that the ratio of drug molecules bound to the melanoproteins in a particular hair sample will be very difficult to determine exactly.

Two major classes of pigments produce the wide range of hair color in mammalian hair, the eumelanins giving dark to brown colors and the pheomelanins giving red and blond shades. Both pigment groups are produced under genetic control in melanocytes in the hair root. Recent studies demonstrated that human hair, whatever the color, contain various portions of both eumelanins and pheomelanins. The ratios show great inter- and intra-individual variability [19–21, 54]. The visual differentiation of hair color does not always reflect the predominant type of melanogenesis in human hair follicles [17, 21, 22, 52 ]. In Caucasian hair the so-called mixed melanogenesis, pheomelanogenesis and eumelanogenesis, is more frequent than pure pheo- or eumelanogenesis. In addition, it is well known that the natural hair color of an individual may not be constant. Normal hair color is altered by different factors, among them, hormonal, nutritional and metabolic influences are largely represented, but also several drugs may affect the color of hair [7, 38, 40].

During the revision of this manuscript a report postulating specific and nonspecific binding of cocaine to hair fragments has been published [23]. Assuming a kind of drug-receptor binding in hair, a competitive binding assay frequently seen in receptor binding studies was used. Drug receptors have traditionally been classified on the basis of the effects that result from their interaction with components of a cell or organism and thereby initiate the biochemical and physiological changes that are characteristic of the response to the drug [66]. It seems unlikely that those events can happen in keratinized tissue.The widely believed a priori assumption that drug soaked hair fibers may serve as a scientific tool to investigate the mechanisms of drug binding to hair is limited to the drug uptake from perspiration such as sweat or sebum and from environmental sources such as pollution. Drug-impregnated keratinized hair samples are unlikely to represent the situation present in hair fibers due to endogenous incorporation of drug molecules into anagen hair follicles and their conservation during keratinization. It should be remembered that the hair follicle and its final product, the keratinized hair fiber, differ completely in morphology, biochemistry, chemical composition and physicochemical behavior [42, 54]. For all the reasons mentioned it seems a real challenge to elucidate drug-melanin binding in hair, which is only one of the variables associated with forensic interpretation in hair analysis. However, the requirement for suitable experimental designs and appropriate models must be stressed, which can only be derived from a profound knowledge of the biology and chemistry of hair as well as from the results of various disciplines.

**Acknowledgement** The project was supported by the Deutsche Forschungsgemeinschaft DFG, Bonn (PO - 474/2–1).

## References


1. Atlasik B, Stepien K, Wilczok T (1980) Interaction of drugs with ocular melanin in vitro. Exp Eye Res 30:325–331
2. Baumgartner WA (1993) Int Pat WO 93//03368
3. Baweja R, Sokoloski TD, Patil PN (1977) Competitive binding between cocaine and various drugs to synthetic levodopa melanin. J Pharm Sci 66:1544–1547
4. Chedekel MR, Murr BL, Zeise L (1992) Melanin standard method: empirical formula. Pigment Cell Res 5:143–147
5. Chen KK, Poth EJ (1929) Racial differences illustrated by mydriatic action of cocaine, eupthalamine and ephedrine. J Pharmacol Exp Ther 36:429–445
6. Cirimele V, Kintz P, Mangin P (1996) Detection and quantification of lorazepam in human hair by GC-MS/NCI in a case of traffic accident. Int J Legal Med 108:265–267
7. Cline DJ (1988) Changes in hair color. Dermatol Clin 6:295–303
8. Cone EJ (1995) Testing for cocaine in hair. In: de Zeeuw RA, Al Hosni I, Al Munthiri S, Maqbool A (eds) Hair analysis - forensic toxicology. Proceedings of the 1995 international conference and workshop. Abu Dhabi, Emirates, 19–23 November, pp 136–160
9. Couper FJ, McIntyre IM, Drummer OH (1995) Extraction of psychotic drugs from human scalp hair. J Forensic Sci 40:83–86
10. Crippa R, Horak V, Prota G, Svoronos P, Wolfram L (1989) Chemistry of melanins. The Alkaloids 36:253–323
11. De Zeeuw RA, Al Hosni I, Al Munthiri S, Maqbool A (1995) Hair analysis - forensic toxicology. Proceedings of the 1995 international conference and workshop. Abu Dhabi, Emirates, 19–23 November, pp 1–501
12. Duchon J, Borovansky J, Hach P (1973) Chemical composition of ten kinds of various melanosomes. Pigment Cell 1:165–170
13. Felix CC, Hyde JS, Sarna T, Sealy RC (1978) Melanin photoreactions in areated media: electron spin resonance evidence for production of superoxide and hydrogen peroxide. Biochem Biophys Res Commun 84:335–341
14. Green SJ, Wilson JF (1996) The effect of hair color on the incorporation of methadone into hair of the rat. J Anal Toxicol 20:121–123
15. Hearing VJ, Lutzner MA (1973) Mammalian melanosomal proteins: characterization by polyacrylamide gel electrophoresis. Yale J Biol Med 46:553–559
16. Henderson GL, Harkey MR, Zou Ch, Jones RT, Jacob P (1996) Incorporation of isotopically labeled cocaine and metabolite into hair: 1. Dose - response relationships. J Anal Toxicol 20: 1–12
17. Inazu M, Mishima Y (1993) Detection of eumelanogenic and pheomelanogenic melanosomes in the same normal human melanocyte. J Invest Dermatol 100:172S–175S
18. Ings RMJ (1984) The melanin binding of drugs and its implications. Drug Metab Rev 15:1183–1212
19. Ito S (1986) Re-examination of the structure of eumelanin. Biochim Biophys Acta 883:155–161
20. Ito S, Jimbow K (1983) Quantitative analysis of eumelanin and pheomelanin in hair and melanomas. J Invest Dermatol 80:268–272
21. Jimbow K, Ishida O, Ito S, Hori Y, Witkop C, King RA (1983) Combined chemical and electron microscopic studies of pheomelanosomes in human red hair. J Invest Dermatol 81:506–511
22. Jimbow K, Fitzpatrick TB, Quevedo WC (1984) Formation, chemical composition and function of melanin pigments. In: Matoltsy AM (ed) Biology of the integument. Springer, New York, pp 278–292
23. Joseph RE, Su TP, Cone EJ (1996) In vitro binding studies of drugs to hair: influence of melanin and lipids on cocaine binding to Caucasoid and African hair. J Anal Toxicol 20:338–344
24. Kidwell DA, Blank DL (1992) Hair analysis: techniques and potential problems. In: Sunshine I (ed) Recent developments in therapeutic drug monitoring and clinical toxicology. Marcel Dekker, New York, p 555–563
25. Kintz P, Traqui A, Mangin P (1992) Detection of drugs in human hair for clinical and forensic applications. Int J Legal Med 105:1–4
26. Kintz P, Traqui A, Mangin P (1993) Pharmacological studies on meprobamate incorporation in human beard hair. Int J Legal Med 105:283–287
27. Kintz P, Cirimele V, Edel Y, Traqui A, Mangin P (1995) Characterization of dextromoramide (Palfium) abuse by hair analysis in a denied case. Int J Legal Med 107:269–272
28. Langmuir I (1916) The constitution and fundamental properties of solids and liquids. J Am Chem Soc 38:2221–2295
29. Larsson B, Tjälve H (1979) Studies on the mechanism of drug-binding to melanin. Biochem Pharmacol 28:1181–1187
30. Larsson B, Olsson S, Szüts T, Ullberg S (1979) Incorporation of [$^{14}$C]nicotine into growing melanin.Toxicol Lett 4:199–203
31. Lindquist NG (1973) Accumulation of drugs on melanin. Acta Radiol Suppl 325:1–92
32. Lyden AB, Larsson B, Lindquist NG (1982) Studies on the melanin affinity of haloperidol. Arch Int Pharmacodyn 259:230–243
33. Maurer HH, Fritz CF (1990) Toxicological detection of pholcodine and its metabolites in urine and hair using radioimmunoassay, fluorescence polarisation immunoassay, enzyme immunoassay and gas chromatography-mass spectrometry. Int J Legal Med 104:43–46
34. Menon IA, Bershad S, Habermann HS, Kurian CJ (1983) A comparative study on the physical and chemical properties of melanin isolated from human black and red hair. J Invest Dermatol 80:202–206
35. Nakahara Y, Simamine M, Takahashi K (1992) Hair analysis for drugs of abuse. III. Movement and stability of methoxyphenamine (as a model compound of methamphetamine) along hair shaft with hair growth. J Anal Toxicol 16:253–257
36. Nakahara Y, Kikura R, Takahashi K (1995) Effect of structural factors on incorporation of drugs into hair - The incorporation rates of amphetamine analogs. In: de Zeeuw RA, Al Hosni I, Al Munthiri S, Maqbool A (eds) Hair analysis - forensic toxicology. Proceedings of the 1995 international conference and workshop. Abu Dhabi, Emirates, 19–23 November, pp 28–49
37. Nakahara Y, Takahashi K, Kikura R (1995) Hair analysis for drugs of abuse. X. Effect of physicochemical properties of drugs on the incorporation rates into hair. Biol Pharm Bull 18: 1223–1227
38. Ortonne JP, Mosher DB, Fitzpatrick TB (1983) Vitiligo and other hypomelanosis of hair and skin. In: Parrish JA, Fitzpatrick TB (eds) Monograph in topics in dermatology. Plenum, New York, p 682
39. Patil PN (1972) Cocaine-binding by the pigmented and nonpigmented iris and its relevance to the mydriatic effect. Invest Ophthalmol Vis Sci 11:739–746
40. Persad S, Menon IA, Habermann HF (1983) Comparison of the effects of UV-visible irradiation of melanins and melanin-hematoporphyrin complexes from human black and red hair. Photochem Photobiol 37:63–68
41. Pötsch L (1994) Zusammenhänge zwischen Haaranalysenergebnis und Haarstruktur. Zentralbl Rechtsmed 42:429
42. Pötsch L (1996) A discourse on human hair fibers and reflections on the conservation of drug molecules. Int J Legal Med 108:285–293
43. Pötsch L, Moeller MR (1996) On pathways for small molecules into and out of human hair fibers. J Forensic Sci 41: 121–125
44. Pötsch L, Skopp G (1996) Stability of opiates in hair fibers after exposure to cosmetic treatment. Forensic Sci Int 81:95–102
45. Pötsch L, Skopp G, Möller MR (1997) Zum Suchtmittelnachweis in Haaren.III. Veränderungen des Opiat-und Kokaingehaltes in Haaren von Drogenkonsumenten nach Blondierung oder Dauerwellbehandlung. Rechtsmedizin (in press)

46. Pötsch L, Skopp G, Möller MR (1997) Biochemical approach on the conservation of drug molecules during hair fiber formation. Forensic Sci Int (in press)
47. Pötsch L, Skopp G, Möller MR (1997) Influence of pigmentation on the codeine content of hair fibers in guinea pigs. J Forensic Sci (in press)
48. Pötsch L, Skopp G, Zörntlein S, Becker J (1997) Zum Suchtmittelnachweis in Haaren. IV. Einfluß der Pigmentierung auf den Ofloxacingehalt in Haaren bei Meerschweinchen. Rechtsmedizin (in press)
49. Potts AM (1962) The concentration of phenothiazines in the eye of experimental animals. Invest Ophthalmol Vis Sci 1:520–530
50. Potts AM (1964) The reaction of uveal pigment in vitro with polycyclic compounds. Invest Ophthalmol Vis Sci 3:405–416
51. Prota G (1980) Recent advances in the chemistry of melanogenesis in mammals. J Invest Dermatol 75:122–127
52. Prota G (1988) Progress in chemistry of melanins and related metabolites. Med Res Rev 8:528–556
53. Reid RW, O'Connor F, Crayton JW (1994) The in vitro differential binding of benzoylegconine to pigmented human hair samples. Clin Toxicol 32:405–410
54. Robbins CR (1988) Chemical and physical behavior of human hair. Springer, New York
55. Rollins DE, Gygi SP, Wilkins DG (1995) Disposition of codeine into hair. In: de Zeeuw RA, Al Hosni I, Al Munthiri S, Maqbool A (eds) Hair analysis - forensic toxicology. Proceedings of the 1995 international conference and workshop. Abu Dhabi, Emirates, 19–23 November, pp 119–135
56. Sachs H, Denk R, Raff I (1993) Determination of dihydrocodeine in hair of opiate addicts by GC/MS. Int J Legal Med 105: 247–250
57. Scatchard G, Coleman JS, Shen CA (1957) Physical chemistry of protein solutions. VII. The binding of some small anions to serum albumin. J Am Chem Soc 79:12–20
58. Shimada K, Baweja R, Sokoloski T, Patil PN (1976) Binding characteristics of drugs to synthetic levodopa melanin. J Pharm Sci 65:1057–1060
59. Skopp G, Pötsch L, Eser HP, Kunze S, Möller MR (1995) Stability of drugs in human hair after exposure to oxidative treatment. In: de Zeeuw RA, Al Hosni I, Al Munthiri S, Maqbool A (eds) Hair analysis - forensic toxicology. Proceedings of the 1995 international conference and workshop. Abu Dhabi, Emirates, 19–23 November, p 494
60. Skopp G, Pötsch L, Aderjan R (1996) Experimental investigations on hair fibers as diffusion bridges and opiates as solutes in solution. J Forensic Sci 41:117–120
61. Swan GA (1974) Structure, chemistry and biosynthesis of the melanins. Fortschr Chem Org Naturst 31:521–556
62. Tjälve H, Nilsson M, Larsson B (1981) Binding of $^{14}$C-spermidine to melanin in vivo and in vitro. Acta Physiol Scand 112:209–214
63. Tjälve H, Nilsson M, Larsson B (1981) Studies on the binding of chlorpromazine and chloroquine to melanin in vivo. Biochem Pharmacol 30:1845–1847
64. Uematsu T, Ohsawa Y, Mizuno A, Nakashima M (1994) Analysis of a new fluoroquinolone derivative (Q-35) in human scalp hair as an index of drug exposure and as a time marker in hair. Int J Legal Med 106:237–243
65. Uematsu T, Sato R, Fujimori O, Nakashima M (1990) Human scalp hair as evidence of individual dosage history of haloperidol; a possible linkage of haloperidol excretion into hair with hair pigmentation. Arch Dermatol Res 282:120–125
66. Voet D, Voet JG (1990) Biochemistry. John Wiley & Sons, New York
67. Wilson JF, Green S, Dunstan DG, Wicks JFC, Brewer C, Perfect N (1995) Relationship between methadone dose, hair methadone concentration and hair color in man. Ther Drug Monit 17:419
68. Zeise L (1991) Bioanalytical studies of melanins. Thesis, The Johns Hopkins University, Baltimore, Maryland