RESEARCH ARTICLES

Program for Evaluating Drug Dissolution Kinetics in Preformulation

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Abstract: The *in vitro* dissolution kinetics of various drugs were studied at different experimental conditions using both a centrically rotating disc method and a modified excentrically rotating disc method. The combination of these two methods provides a preformulation program that includes many important pharmaceutical and biopharmaceutical parameters related to drug dissolution. The influence of the hydrodynamic conditions on the dissolution rate was demonstrated and the intrinsic dissolution tendency was obtained. Further, the acid dissociation constant, diffusion coefficient and enthalpy of dissolution can be calculated from data obtained from the rotating disc experiments. The relationship between dissolution rate and aqueous solubility of each drug tested is also considered.

At preformulation, several tests are conducted on a new drug compound, in order to optimize those physical and chemical properties that are considered important for the formulation of a stable, effective and safe dosage form (1, 2). Among the tests available, solubility and dissolution rate experiments at various conditions can provide important information about biopharmaceutical issues, since the drug must first dissolve in the gastro-intestinal fluids in order to be absorbed. It has been reported that some drugs such as digoxin, various erythromycin esters and different hydrates of ampicillin display dissolution rate limited absorption (3–6). Kaplan reported that the dissolution rate should be higher than 1 mg/(cm^2 · min) in order to avoid problems with the extent of bioavailability (7). However, this value must be treated with caution, since the dissolution rate is highly dependent on hydrodynamic conditions and pH. The dissolution kinetics of different drugs at various experimental conditions were studied in several reports with the use of rotating compressed discs (8–16). In order to obtain meaningful values of the dissolution rate, the experimental conditions need to be well defined. The hydrodynamic theory derived by Levich for centrically rotating discs proved to be useful (11, 17). While it was slightly modified by Riddiford (18), the theory of Levich can be considered applicable to most practical purposes.

The aim of the present paper is to present a program for characterizing *in vitro* dissolution kinetics at preformulation in terms of both diffusion controlled dissolution kinetics and initial mass transfer from solid to liquid phase, i. e. intrinsic rate of dissolution. It is also shown that the data obtained from the dissolution experiments can be used to calculate other important constants such as diffusion coefficients, pK_a, enthalpies of dissolution and aqueous solubilities.

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Materials and Methods

Chemicals

Acetylsalicylic acid, pentobarbital, trimethoprim and sulfamethizole of commercial grades were used. Alaproclate hydrochloride monohydrate, remoxipride hydrochloride monohydrate, raclopride tartrate, buciclovir and buciclovir hydrochloride, (Astra Läkemedel AB, Sweden) were used as obtained. To prepare the buffer solutions, HCl, NaCl, H_3PO_4, NaH_2PO_4, Na_2HPO_4 of analytical grade were used.

Diffusion controlled dissolution rates

Dissolution rates were determined in water at 37°C from centrically mounted compressed discs as a function of angular velocity (Fig. 1). The preparation of the discs and the experimental procedure have been described elsewhere (8). While

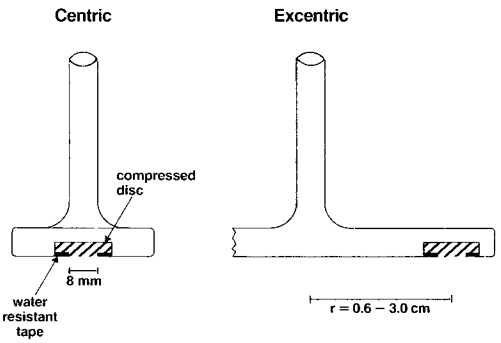

Fig. 1 Different mountings for rotating disc systems.

rotating, the discs were lowered into 200 ml water, and the dissolution medium was analyzed spectrophotometrically during the first minute of release. The observed dissolution rates were calculated by linear regression analysis from the relationship between amount dissolved per cm^2 and time. The dissolution properties were studied at various rotating speeds of the discs (200–1600 rpm), and the data are presented according to the Levich theory, i. e.,

$$G = 0.62 \cdot D^{2/3} \cdot \nu^{-1/6} \cdot C_s \cdot \omega^{1/2} \quad (1)$$

Thus, a plot of G *versus* $\omega^{1/2}$ (n=5) gives a straight line from which the observed diffusion controlled dissolution rate can be calculated at any relevant rotating speed, provided that laminar flow is present in the system. The experimental set-up is demonstrated in Fig. 2.

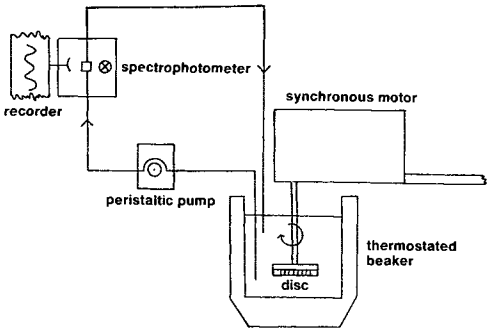

Fig. 2 Experimental set-up for the determination of dissolution rates from a rotating disc.

Dissolution and pH

Diffusion controlled dissolution rates were determined at 1000 rpm as a function of bulk pH. The acid dissociation constants were determined according to general solubility-pH theory using a non-linear least squares computer program (8).

Dissolution and temperature

The diffusion controlled dissolution rates were determined in water at 100 rpm from centrically mounted discs as a function of temperature. By plotting the logarithm of the dissolution rates as a function of the reciprocal of the absolute temperature according to Arrhenius it is possible to estimate the enthalpies of dissolution at a given temperature (19).

Intrinsic rate of dissolution

The intrinsic rate of dissolution expressed as the initial release from solid to aqueous phase without a diffusion process present can be obtained if the experimental conditions are arranged in such a way that the rotating disc is always in contact with fresh solvent. Such conditions could never be properly achieved if the disc is kept at a small constant distance from the centre of rotation and only the speed of revolution is increased. However, if the distance between the disc and the rotating support could also be varied, the values of the dissolution process corresponding to an infinite distance would be the intrinsic rate of dissolution. The rationale of this has been previously discussed (9, 10). The dissolution rate model for obtaining the intrinsic rate of dissolution for a laminar flow system is shown in eq. (2),

$$\frac{1}{G} = \frac{1}{k_i} + \frac{k}{r\sqrt{\omega}} \qquad (2)$$

If 1/G is plotted *versus* $1/(r\sqrt{\omega})$ a straight line is obtained which for $1/(r\sqrt{\omega}) = 0$ extrapolates to $1/k_1$, i. e. the reciprocal of the intrinsic rate of dissolution. The rotating support for these experiments is shown in Fig. 1 (cf. excentric mounting).

Diffusion coefficients

Diffusion coefficients were determined in water at 37° C using a diffusion cell with a SPECTRAPOR 2 membrane (Spectrum Medical Industries Inc. USA). A 10 ml portion of drug solution was placed in the upper compartment and the drug was allowed to diffuse through the membrane into 86 ml of sink which was gently agitated with a magnetic stirrer. The sink was continuously analyzed spectrophotometrically using a peristaltic pump and a flow cuvette. The diffusion coefficients were evaluated according to (20).

Diffusion coefficients were also calculated from the dissolution experiments applying the Levich relationship [cf. eq (*1*)] and the values were compared with the observed ones. A kinematic viscosity (ν) of $6.964 \cdot 10^{-3}$ cm^2/s was applied.

Solubilities

Drug in excess, to form saturated solutions, was added to buffer solutions or water thermostated at 37° C. The resulting suspensions were equilibrated using a magnetic stirrer for 24 hours. Samples of 5 ml were filtered through 0.1 μm polycarbonate filters (NUCLEPORE Co.) and diluted to suitable concentrations for spectrophotometry.

The solubilities were also calculated from the dissolution rate experiments for centrically rotating discs by applying a general solubility-dissolution rate relationship for a variety of chemicals.

Preformulation Program

The suggested preformulation program for dissolution testing is given below.

1. Determination of the dissolution rate as a function of hydrodynamics in a laminar flow system according to Levich (17) and determination of the diffusion coefficient.
2. Determination of the intrinsic tendency for a compound to dissolve, i. e. the initial mass transfer from solid to aqueous phase, without a diffusion controlled transport present (intrinsic rate of dissolution).
3. Determination of the influence of pH on the diffusion controlled dissolution rate (Levich model). Determination of pK_a values.
4. Determination of the influence of temperature on the diffusion controlled dissolution rate. Determination of the enthalpy of dissolution at a given temperature.
5. Determination of the aqueous solubilities both from equilibrium tests and from dissolution experiments in order to determine whether a change in crystal form occurs during the solvatization process.

In this study, water (37° C) is used as dissolution medium except when the pH dependency is investigated. However, other solvents or buffers may be used depending on the specific aim of the test.

Results

Diffusion controlled dissolution

Figure 3 depicts the observed diffusion controlled dissolution rates in water (37° C) as a function of angular velocity for three different compounds. Straight lines are obtained, and the intercepts are very close to origo which agrees with the theory of Levich (17). Mooney et al. (11) reported that a non-zero intercept was found for indomethacin indicating the conditions for a static disc. This was not seen for the compounds reported in this paper. Table I summarizes the slopes obtained for the various drug compounds. On the basis of eq. (1) the diffusion

coefficient can be calculated from the slope of G *versus* $\omega^{1/2}$. There is a fairly good agreement between the calculated diffusion coefficients obtained from the Levich relationship and the observed values obtained by diffusion cell measurements as shown in Table I. This result validates the assumption that our experimental procedure yields a laminar flow near the rotating disc surfaces. The corresponding Reynolds numbers were calculated to be $4.8 \cdot 10^2$ and $3.8 \cdot 10^3$ at the lowest and highest angular velocity, respectively.

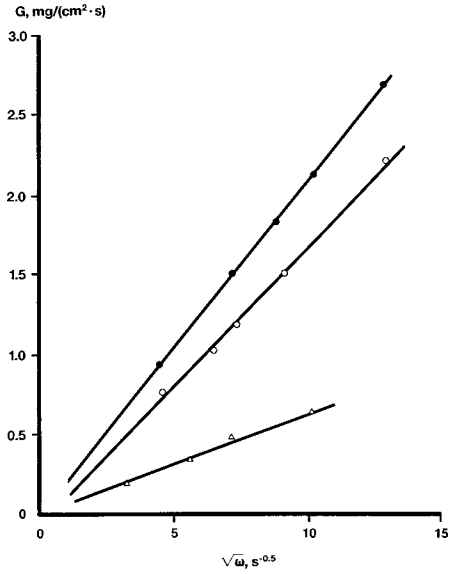

Fig. 3 Observed dissolution rates in water 37° C from centrically rotating discs as a function of the squareroot of the angular velocity; (●) buciclovir hydrochloride (○) remoxipride hydrochloride monohydrate (△) alaproclate hydrochloride monohydrate.

Table I. Slopes ($mg \cdot cm^{-2} \cdot s^{-0.5}$) and correlation coefficients (r) calculated from the Levich relationship according to eq. (*1*), diffusion coefficients obtained either by applying eq. (*1*), D_{diss}, or from diffusion cell measurements, D_{obs}, and aqueous solubilities, C_s, water 37° C.

Drug	Slope	r	$D_{diss.}$	$D_{obs.}$	Cs
Pentobarbital	$2.13 \cdot 10^{-4}$	0.997	$5.2 \cdot 10^{-6}$	–	0.5
Sulfamethizole	$4.93 \cdot 10^{-3}$	0.998	$7.0 \cdot 10^{-6}$	$6.1 \cdot 10^{-6}$	0.95
Buciclovir	$2.30 \cdot 10^{-3}$	0.967	$7.9 \cdot 10^{-6}$	–	4.1
Acetylsalicylic acid	$4.48 \cdot 10^{-3}$	1.000	$8.6 \cdot 10^{-6}$	$3.2 \cdot 10^{-6}$	7.5
Alaproclate hydrochloride monohydrate	$4.85 \cdot 10^{-2}$	0.992	$4.3 \cdot 10^{-6}$	$8.0 \cdot 10^{-6}$	130
Raclopride tartrate	0.177	0.982	$12.3 \cdot 10^{-6}$	–	234
Remoxipride hydrochloride monohydrate	0.186	0.998	$6.5 \cdot 10^{-6}$	$9.9 \cdot 10^{-6}$	375
Buciclovir hydrochloride	0.210	0.999	$11.0 \cdot 10^{-6}$	–	294

Intrinsic rate of dissolution

The intrinsic rate of dissolution is interpreted as the initial release of drug molecules from the solid surface into fresh solvent. Fig. 4 shows a plot of 1/G *versus* $1/(r\sqrt{\omega})$ according to eq. (2) for remoxipride hydrochloride monohydrate in water (37° C). The intercept in Fig. 4 is equal to the reciprocal of the intrinsic dissolution rate of remoxipride. Theoretically, it is obtained at a condition where the solvent flow rate past the disc surface is infinitely high. Table II summarizes the intrinsic rates of dissolution for the different compounds. The results in Table II clearly demonstrate that the dissolution tendency varies for different chemical entities probably because of differences in energy structure between the solid and the liquid phase for each compound. The fact that different drugs dissolve with various rates can therefore not only be explained by differences in diffusion properties or thickness of the diffusion boundary layer but also by differences in solubility.

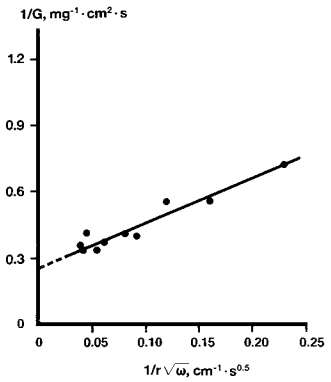

Fig. 4 Plot of the reciprocal of the observed dissolution rates *versus* the reciprocal of the distance from the rotating centre and the squareroot of the angular velocity for remoxipride hydrochloride monohydrate in water 37° C. The intercept denotes the reciprocal value of the intrinsic rate of dissolution.

Table II. Intrinsic rates of dissolution in water at 37° C calculated from eq. (*2*).

Drug	Intrinsic rate of dissolution $mg/(cm^2 \cdot s) \pm$ S.E.M.
Sulfamethizole	0.008 ± 0.00067
Buciclovir	0.04 ± 0.004
Acetylsalicylic acid	0.07 ± 0.006
Alaproclate hydrochloride monohydrate	1.38 ± 0.050
Raclopride tartrate	2.22 ± 0.403
Remoxipride hydrochloride monohydrate	3.90 ± 0.120
Buciclovir hydrochloride	4.45 ± 0.650

pH

For protolytic drug compounds, the influence of pH on the dissolution properties is important, since it can influence the absorption from the gastro-intestinal tract. Figure 5 shows a pH-dissolution rate profile for buciclovir. The solid line in Fig. 5 denotes the calculated regression line. From this plot, it is possible to calculate the acid dissociation constants with the use of a non-linear least squares computer program. Numerical data are shown in Table III for buciclovir and several other drugs. The pK_a values for acetylsalicylic acid, pentobarbital, trimethoprim, alaproclate and sulfamethizole have been reported elsewhere applying the same analytical technique (8).

Solubility

Figure 6 shows a general relationship between the aqueous solubilities and the observed dissolution rates for the substances investigated in this paper. The regression line was cal-

culated to be log C_s = 0.92(±0.055) · log G + 0.74(+0.0718) ±S.E.M., n = 8. Similar relationships have been previously documented (8, 19, 21, 22). The dissolution rates were determined from centrically mounted discs at a rotating speed of 500 rpm with water (37° C) as the dissolution medium. Thus, if a limited drug quantity is available initially, it is possible to predict with good precision the order of magnitude of the equilibrium concentration from a rapid *in vitro* dissolution experiment, using the rotating disc principle.

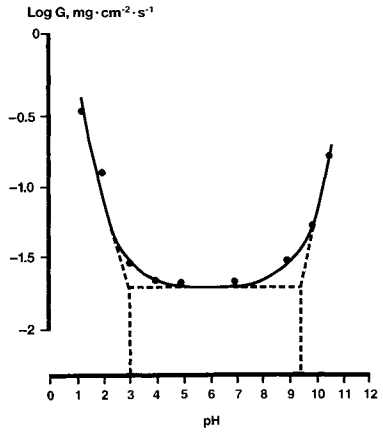

Fig. 5 Log dissolution rate *versus* pH for buciclovir at 37° C. The dissolution rates were determined from centrically mounted discs at 1000 rpm.

Table III. Dissociation constants determined from rotating disc experiments.

Compound	pKa_1	pKa_2
Acetylsalicylic acid	3.6[1)]	
Trimethoprim	7.1[1)]	
Pentobarbital	8.1[1)]	
Sulfamethizole	2.1[1)]	5.1
Buciclovir	3.0	9.5
Raclopride tartrate	6.7	9.6

[1)] taken from ref. (8)

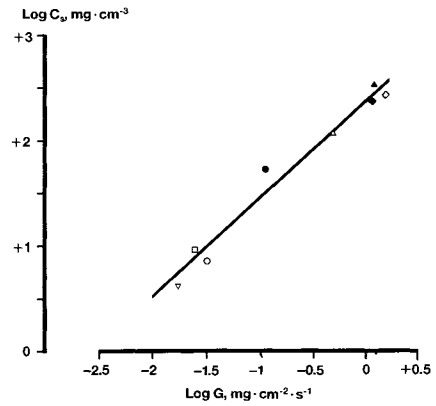

Fig. 6 A general log-log relationship at 37° C between equilibrium concentrations and dissolution rates determined from centrically rotating discs (500 rpm.). (△) Alaproclate hydrochloride monohydrate; (□) sulfamethizole; (●) acetylsalicylic acid; (○) trimethoprim; (▲) remoxipride hydrochloride monohydrate; (▽) buciclovir; (◇) buciclovir hydrochloride; (◆) raclopride tartrate.

Temperature

A number of papers describe the influence of temperature on the dissolution rate (19, 23–26). For some compounds such as aminocephalosporins, a comparison between the heat of solubility and the heat of dissolution indicated that the release of these substances was diffusion controlled (23). Figure 7 shows Arrhenius plots for sulfamethizole and acetylsalicylic acid from which it is possible to calculate the activation energy for the diffusion controlled dissolution which in turn can be expressed as the enthalpy of dissolution using the classical equation,

$$E_a = \Delta H^{\ddagger} + RT. \quad (3)$$

The enthalpy values for sulfamethizole and acetylsalicylic acid for a diffusion controlled dissolution process have been reported (19). From such relationships it is possible to calculate the dissolution kinetics at any temperature within the studied range.

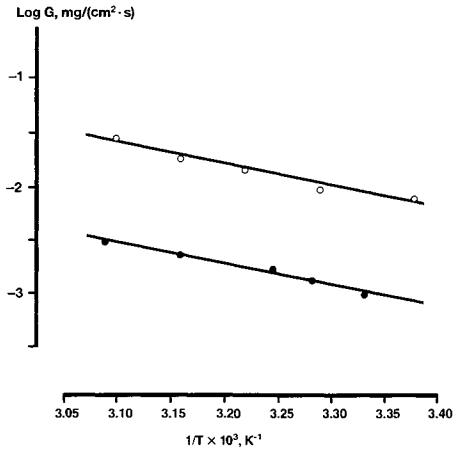

Fig. 7 Arrhenius plots of the dissolution rates at 100 rpm in water (○) acetylsalicylic acid; (●) sulfamethizole.

List of symbols

- G = observed dissolution rate at a given speed of rotation, mg/(cm² · s)
- D = diffusion coefficient, cm²/s
- C_s = aqueous solubility, mg/cm³
- r = distance from the centre of the rotating support to the disc surface as shown in Fig. 1, cm
- ν = kinematic viscosity, cm²/s
- ω = angular velocity, s^{-1}
- k_1 = intrinsic rate of dissolution, mg/(cm² · s)
- k = a proportionality constant
- E_a = activation energy, kJ/mol
- $\Delta H^{\ddagger}$ = enthalpy of dissolution, kJ/mol
- T = absolute temperature, K
- R = 8.314 J/(mol × K)

References

(1) Shami, E. G., Dudzinski, J. R., Lantz, R. J. (1976) in The Theory and Practice of Industrial Pharmacy (Lachman, L., Lieberman, H. A. and Kanig, J. L., ed.). pp. 1–31, Lea & Febiger, Philadelphia.

(2) Graffner, C., Johansson, M. E., Nicklasson, M., Nyqvist H. (1985) J. Pharm. Sci. 74, 16–20.

(3) Lindenbaum, J., and Butler, V. P. (1973) The Lancet, 2, 1215–1217.

(4) Nelson, E. (1962) Chem. Pharm. Bull. 10, 1099–1101.
(5) Poole, J. W., Owen, G., Silverio, J., Freyhof, J. N., Rosenman, S. B. (1968) Curr. Ther. Res. 10, 292–303.
(6) Hill, S. A., Jones, K. H. Seager, H. Taskis, C. B. (1975) J. Pharm. Pharmac. 27, 594–598.
(7) Kaplan, S. A. (1972) Drug Metab. Rev. 1, 15–33.
(8) Nicklasson, M., Brodin, A., Nyqvist, H. (1981) Acta Pharm. Suec. 18, 119–128.
(9) Nicklasson, M., Brodin, A., Sundelöf, L.-O. (1982) Acta Pharm. Suec. 19, 109–118.
(10) Nicklasson, M., Brodin, A., Sundelöf, L.-O. (1983) Int. J. Pharm. 15, 87–95.
(11) Mooney, K. G., Mintun, M. A., Himmelstein, K. J., Stella, V. J. (1981) J. Pharm. Sci. 70, 13–22.
(12) Singh, P., Desai, S. J., Flanagan, D. R., Simonelli, A. P., Higuchi, W. I. (1968) J. Pharm. Sci. 57, 959–965.
(13) Virtsava, L. A., Dzelme, Y. R., Tiliks, Y. E., Bugarenko, L. T. (1978) Russ. J. Phys. Chem. 52, 1638–1641.
(14) Levy, G., Tanski, W. (1964) J. Pharm. Sci 53, 679.
(15) Wood, J. H., Syarto, J. E., Letterman, H. (1965) J. Pharm. Sci. 54, 1068.
(16) Prakongpan, S., Higuchi, W. I., Kwan, K. H., Molokhia, A. M. (1976) J. Pharm. Sci. 65, 685–689.
(17) Levich, V. G. (1962) in Physicochemical hydrodynamics p. 60, Prentice-Hall, Englewood Cliffs, N. J.
(18) Riddiford, A. C. (1963) in Advances in electrochemistry and electrochemical engineering. (Delahay, P. ed.) pp. 47–117. Interscience Publishers, New York.
(19) Nicklasson, M., Brodin, A., Stenlander, C. (1982) Acta Pharm. Suec. 19, 25–36.
(20) Higuchi, W. I., (1962) J. Pharm. Sci. 51, 802–804.
(21) Hamlin, W. E., Northam, J. I., Wagner, J. G. (1965) J. Pharm. Sci. 54, 1651-1653.
(22) Nelson, K. G., Shah, A. C. (1975) J. Pharm. Sci. 64, 610–614.
(23) Tsuji, A., Nakashima, E., Hamano, S., Yamana, T. (1978) J. Pharm. Sci. 67, 1059–1066.
(24) Tsuji, A., Nakashima, E., Yamana, T. (1979) J. Pharm. Sci. 68, 308–311.
(25) Cooper, A. R., Kingery, W. D. (1962) J. Phys. Chem. 66, 665–669.
(26) Nogami, H., Nagai, T., Suzuki, A. (1966) Chem. Pharm. Bull. 14, 329–338.

Synthesis and Pharmacological Characterization of Fluorescent Opioid Receptor Probes

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Abstract: A series of five fluorescent opioid receptor probes was synthesized by coupling naloxone, oxymorphone or naltrexone with fluorescein or tetramethylrhodamine B. The series was characterized for capacity to displace ^{3}H-dihydromorphine from rat brain opioid receptors. All compounds showed receptor binding, and 1-(*N*)-fluoresceinyl naltrexone thiosemicarbazone displayed the highest mu-receptor affinity with a K_i value of 3 nM. 1-(*N*)-fluoresceinyl naloxone thiosemicarbazone was a morphine antagonist *in vivo*, approximately 6 % as potent as naloxone and naloxonazine in the mouse hot-plate test.

Opioid receptors have been studied extensively with radioactive tracer ligand displacement assays. These techniques are limited in some aspects, for instance they do not allow studies of fast kinetics of receptor-ligand interactions or direct visualization of receptor distribution in tissues. Fluorescent opioid probes might be alternative tools with particular advantages for these purposes and also for flow cytometric sorting of opioid receptor bearing membrane vesicles or cells. Fluorescent opioid peptides and a dansylated naloxone derivative have been reported previously (1, 2, 3). We have synthesized a series of morphinan-type fluorescent compounds for investigation of their properties as potential opioid receptor probes. A preliminary report on the synthesis, identification, and activities of four of these probes has appeared (4). Some probes have shown alterations in efficacy in comparison to their parent compounds (5) and prolonged duration of *in vitro* receptor blockade (6,7). The type of chemical coupling between opioid and fluorescent units was varied in order to compare chemical stability and duration of effects.

We report here the synthesis of all five probes, describe some stereochemical features as determined via high-resolution ^{1}H-NMR studies, and compare all probes for their ability to displace ^{3}H-dihydromorphine from rat brain membranes. Also, 1-(*N*)-fluoresceinyl naloxone thiosemicarbazone, the probe with the highest antagonistic activity on the guinea-pig myenteric plexus-longitudinal muscle (5) was compared to naloxone and naloxonazine for morphine antagonism in the mouse hot-plate test. Naloxonazine has been introduced as a long-acting opioid antagonist (8) and was included for comparison of duration of effects.

Materials and Methods

The TLC was carried out on E. Merck aluminum-supported sheets (precoated TLC sheets, silica gel 60F-254, layer thickness 0.2 mm, catalogue no. 5539). The two eluents used were: System I: $CHCl_3$: MeOH: conc. NH_4OH = 135:10:2 (v/v), and

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