Spasmolytics IV: Azo and Azoxy Derivatives of 3-Tropanyl 2,3-Diarylacrylates

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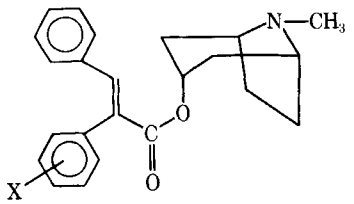
Abstract □ Some derivatives of potent spasmolytic aminoesters were made and tested to determine if they were satisfactory prodrug forms. These azo- and azoxyesters were much less active than the aminoesters in a 5-hr. spasmolytic test in mice, and results suggest that these potential aminoesters are not satisfactory prodrugs.

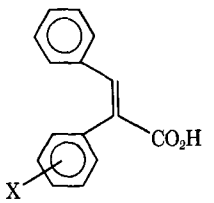
Keyphrases □ 3-Tropanyl 2,3-diarylacrylates, azo and azoxy derivatives—synthesis, evaluation as potential spasmolytic prodrug forms □ Spasmolytics, synthesis, evaluation—3-tropanyl 2,3-diarylacrylate derivatives

It was recently reported that a series of 3-tropanyl 2,3-diarylacrylates possessed spasmolytic activity without anticholinergic effect (1). Spasmolytic test data for nine of the first 10 of the 2-aryl-3-phenyl esters were subjected to multiple-parameter analysis[1]. It was found that the linear equation: $-\log ED_{50} = -1.624\ \sigma_o - 0.796$, where σ_o refers to the electron density in the 2-phenyl ring at the position *ortho* to the side chain, accounted for 95% of the variability in the test data for seven of the compounds. In contrast, the linear equation: $-\log ED_{50} = -1.077\ \sigma_{sc} - 0.906$, where σ_{sc} refers to the electron density in the 2-phenyl ring at the carbon attached to the side chain, accounted for only 54% of the variability in the test data for the seven compounds.



I: X = 4-NO_2
II: X = 4-NH_2
III: X = 3-NO_2
IV: X = 3-NH_2
V: X = 3-N=N-3
VI: X = 3-N=N-3 (↓ O)

XII: X = 4-N=N-4
XIII: X = 4-N=N-4 (↓ O)
XIV: X = 4-N=N-φ
XV: X = 3-N=N-φ
XVI: X = 4-OH, 3-N=N-φ



VII: X = 3-NO_2
VIII: X = 4-NO_2
IX: X = 3-N=N-3
X: X = 3-N=N-3 (↓ O)
XI: X = 4-N=N-4

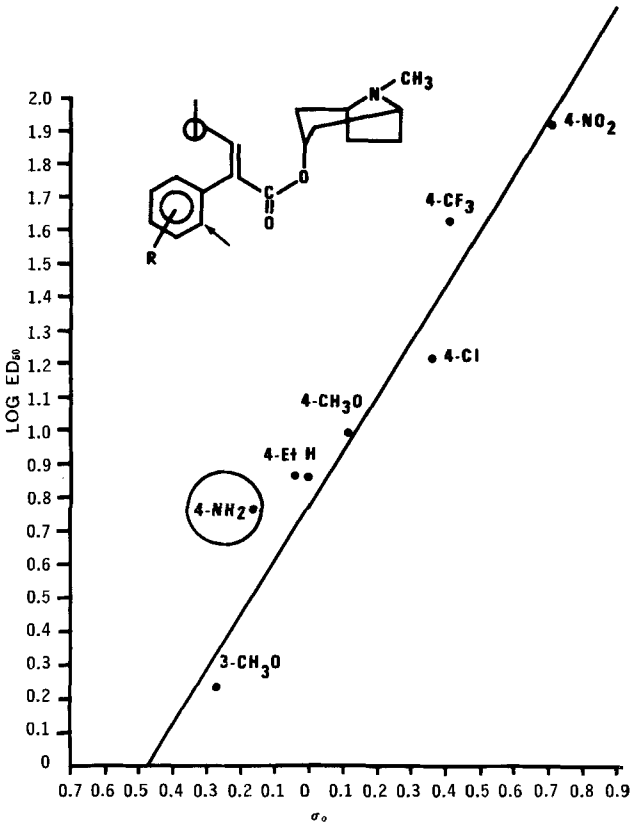


Figure 1—*Relationship between spasmolytic potency and sigma at the* ortho*-position (arrow). The circled compound provided a test of this relationship. Its actual potency was close to the potency predicted by the line.*

A plot of σ_o *versus* ED_{50} (Fig. 1) shows that compounds with negative σ_o values are more potent than those with positive σ_o values. Test results for the 4-amino compound available after the Hansch analysis provided a test of the ability of the equation to predict activity. It fell very close to the predicted curve. Figure 1 suggests that compounds with more highly negative σ_o values will be more potent.

To follow this lead, the 3-amino, 3-isobutylamino, and 3-hydroxy compounds were made. Subsequent to this work, data on these and certain newer com-

[1] By Professor C. Hansch.

Table I—Azo and Azoxy Compounds

	V	VI	IX	X				
	←M+→						Azo-benzene[a]	Azoxy-benzene[b]
	720	736	474	490	XII	XIII		
λEtOH (log $E_{max.}$)	220(4.56) 288(4.63) 440(2.73)	220(4.55) 286(4.63) 440(0)	274(4.55) 440(2.85)	272(4.53) 440(0)	223(4.42) 234(shoulder) 282(4.54) 344(4.38) 440(3.08)	221(shoulder) 282(4.60) 342(4.56) 440(0)	230(4.2) 320(4.3) 438(2.9)	231(3.92) 260(3.85) 323(4.16)
Color	Yellow-orange	Light-yellow	Yellow-orange	Tan	Orange	Yellow	Orange-red	Pale-yellow
Type	3-Azoester	3-Azoxyester	3-Azoacid	3-Azoxyacid	4-Azoester	4-Azoxyester	Azo	Azoxy

[a] W. S. McGuire, T. F. Izzo, and S. Zuffanti, *J. Org. Chem.*, **21**, 632(1956). [b] P. H. Gore and O. H. Wheeler, *J. Amer. Chem. Soc.*, **78**, 2160(1956).

pounds in the series were analyzed and it was found that the equation: $\log 1/c = 4.744 - 1.215\ \sigma_o^2 - 0.842\ \sigma_o$, accounted for 86% of the test data for 14 of the compounds. A plot of $\log 1/c$ *versus* σ_o gave a parabola with an ideal σ_o of −0.346, which suggested that the two most potent compounds in the series had been made (2). These compounds, 3-tropanyl 2-(2- and 3-methoxyphenyl)-3-phenylacrylate, were as potent as atropine in the fecal pellet inhibition test.

The purposes of this article are to: (*a*) describe the chemistry that led to the 3-amino compound and to some potential precursor amines, and (*b*) report pharmacological test results on these compounds.

DISCUSSION

The potent spasmolytic, 3-tropanyl 2-(4-aminophenyl)-3-phenylacrylate hydrochloride hydrate (II), was first made in 56% yield by hydrogenation of the 4-nitroester hydrochloride hydrate (I) over platinum on charcoal. The same procedure was used to reduce the isomeric 3-nitroester hydrochloride (III) to give a hydrated compound in low yield with identical elemental composition as II; this was assumed to be 3-tropanyl 2-(3-aminophenyl)-3-phenylacrylate hydrochloride hydrate (IV). Initial test results looked interesting, so more tests were planned; for these, more supplies were required. This time the reduction was run over Raney nickel in an attempt to improve the yield. But the two reduction products of III were different. The original melted at 283.5–284.5° dec. while the latter melted at 230–231° dec. (1). NMR showed the original had no NH protons, while the latter had two NH protons. Thus, the latter was established as the authentic IV, and the original was probably an azoester (V) or azoxyester (VI).

Authentic V was made by the sequence: nitroacid (VII) → azoacid (IX) → azoester (V), and VII (1 mole) was reduced with zinc dust (2 moles) and sodium hydroxide (5 moles) by the procedure for nitrobenzene → azobenzene (3). But the product was the azoxyacid (X) instead of IX. When the nitroacid, zinc dust, and sodium hydroxide ratios were changed to 1, 4, and 9.6, respectively, the hydrazoacid was obtained and air oxidized (4) to the azoacid (IX), which was converted to the authentic azoester (V), m.p. 288–289° dec., M^+ 720. The original reduction product was assigned Structure VI because it had M^+ of 736 and had no UV absorption at 440 nm. (azo band). Additional data are listed in Table I. VI was subsequently made in 67% yield by reducing the free base III over platinum on carbon.

The formation of IV, V, and VI from III in alkaline media is explained in Scheme I (5). Evidence in the literature suggests that a similar reductive sequence might occur *in vivo*. For example, *m*-nitrosonitrobenzene (RNO), *m*-nitrophenylhydroxylamine (RNHOH), and *m*-nitroaniline (RNH_2) are found in the urine of rabbits dosed with *m*-dinitrobenzene (6). Azoxy compounds were isolated in the urine of man and rabbits, although these might be artifacts which appear on standing or during isolation procedures because aromatic hydroxyamines are rapidly converted to azoxy compounds in neutral or alkaline solution (7). Azo compounds are reduced *in vivo* to hydrazo compounds, which are cleaved to amino compounds (8, 9). Salicylazosulfapyridine is an example (10). Also a variety of aromatic nitro compounds, including chloramphenicol, are reduced to the corresponding aromatic amines (11).

Thus, the nitro, azoxy, and azo compounds might be active *per se* or they might be active only after *in vivo* conversion to the amino compound. In either case, the authors wanted to pursue this in the *m* and *p* series and in related compounds.

The isomeric 4-azoester (XII) was made by the sequence: nitroacid (VIII) → azoacid (XI) → azoester (XII), and the 4-azoxyester (XIII) was made by reducing the 4-nitroester base (II). The azophenylesters (XIV and XV) were made from the aminoesters (II and IV) and nitrosobenzene (12). The benzeneazophenol ester (XVI) was made from the phenol ester and benzenediazonium chloride (13).

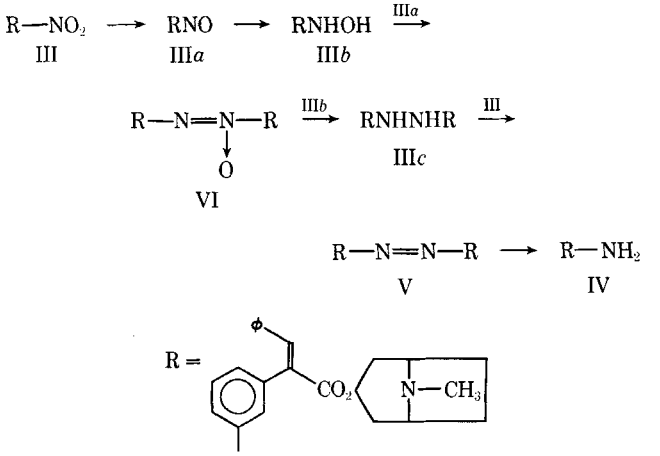
EXPERIMENTAL

Chemistry—Melting points were determined in open capillary tubes, using the Thomas-Hoover Uni-Melt, and are uncorrected.

3,3'-Diphenyl-2,2'-[azoxydi-(m-phenylene)]-diacrylic Acid (X)—This was made by the general method Bigelow and Robinson (3) used to prepare azobenzene. 2-(*m*-Nitrophenyl)-3-phenylacrylic acid (VII, 5.39 g., 0.02 mole) was suspended in methanol (35 ml.), and NaOH (4.04 g., 0.10 mole) in water (8 ml.) was added. Zinc dust (2.68 g., 0.041 mole) was added, and the mixture was heated and stirred at reflux for 11 hr. The solution was filtered, and the filtrate was acidified with hydrochloric acid. Crystallization (dioxane–water) gave 4.4 g. (90%) of a tan solid, m.p. 288–290° dec.

Anal.—Calc. for $C_{30}H_{22}N_2O_5$: C, 73.46; H, 4.52; N, 5.71. Found: C, 73.73; H, 4.70; N, 5.64.

3,3'-Diphenyl-2,2'-[azodi-(m-phenylene)]-diacrylic Acid (IX)—This was made by the general method of Bigelow and Robinson (3), but the nitro compound–zinc–sodium hydroxide ratio was changed from 1:2:5 to 1:4:9.6. 2-(*m*-Nitrophenyl)-3-phenylacrylic acid (VII, 20.2 g., 0.075 mole) was suspended in methanol (200 ml.), and NaOH (28.8 g., 0.72 mole) in water (60 ml.) was added to form a solution. Zinc dust (10 g.) was added, and the



Scheme I

Table II—Lethal, Spasmolytic, and Mydriatic[a] Effects in Mice of 3-Tropanyl 2,3-Diarylacrylates (Dose, mg./kg. p.o.)

Compound (Free Base)	X	MLD	Fecal Pellet, ED_{50}
I[b]	4—NO_2	162	82
II[b]	4—NH_2	512	5.7
III	3—NO_2	—	—
IV[b]	3—NH_2	256	8.2
V	3—N=N—3	256	>63
VI	3—N=N—3 (↓ O)	>1024	52
XII	4—N=N—4	768	>63
XIII	4—N=N—4 (↓ O)	>1024	>100
XIV	4—N=N—ϕ	>1024	82
XV	3—N=N—ϕ	768	60
XVI	4—OH, 3—N=N—ϕ	>1024	46
Atropine[b]	—	620	2.5

[a] Only atropine produced mydriasis. [b] These data were reported in *Reference 1*.

mixture was heated and stirred at reflux. After 1 hr., additional zinc dust (9.6 g.) was added; the blood-red solution turned orange and then pale yellow-green in 2.5 hr. The hydrazoacid solution was filtered, and air was passed through the warmed filtrate for about 20 hr. until the solution was red and the azoacid salt started to precipitate (4). The mixture was cooled, the orange precipitate was dissolved in aqueous methanol, and the solution was acidified with hydrochloric acid. Crystallization from dioxane–water gave, after drying *in vacuo*, 12 g. (68%) of a yellow-orange product, m.p. 299.5–300.5° dec.

Anal.—Calc. for $C_{30}H_{22}N_2O_4$: C, 75.94; H, 4.67; N, 5.90. Found: C, 75.76; H, 4.55; N, 5.73.

*3,3'-Diphenyl-2,2'-[azodi-(*p*-phenylene)]-diacrylic Acid (XI)*—This was made from 2-(*p*-nitrophenyl)-3-phenylacrylic acid (VIII, 11 g.) by the method described for IX. Crystallization from methanol–water gave 65% of a tan product, m.p. 329° dec.

Anal.—Calc. for $C_{30}H_{22}N_2O_4$: C, 75.94; H, 4.67; N, 5.90. Found: C, 75.58; H, 4.97; N, 5.83.

3,3'-Diphenyl-2,2'-[azodi-(m-phenylene)]-diacrylic Acid, Bis(3-tropanyl) Ester Dihydrochloride, Dihydrate (V)—A mixture of IX (5.6 g.), thionyl chloride (7 g.), dry benzene (56 ml.) and pyridine (1 drop) was heated at reflux for 2 hr. Excess thionyl chloride and benzene were removed, and three separate portions of dry benzene were added and removed in the same way. The residue was dissolved in dry benzene (60 ml.), and dry tropine (8.33 g.) in dry benzene (55 ml.) was added slowly with stirring. The mixture was heated at reflux temperature for 24 hr. and filtered. The filtrate was washed with water until neutral and dried, and the solvent was removed. The residue was dissolved in methanol, and excess methanolic HCl was added. Crystallization from ethanol gave 4.9 g. (51%) of an orange product, m.p. 288–289° dec.

Anal.—Calc. for $C_{46}H_{48}N_4O_4 \cdot 2HCl \cdot 2H_2O$: C, 66.58; H, 6.56; N, 6.75; Cl, 8.55. Found: C, 66.44; H, 6.61; N, 6.78; Cl, 8.37.

3,3'-Diphenyl-2,2'-[azodi-(p-phenylene)]-diacrylic Acid, Bis(3-tropanyl) Ester Dihydrochloride, 1.5 Hydrate (XII)—This was made from XI (6 g.) by the method described for V. Crystallization from methanol–isopropanol gave an orange solid (39% yield), m.p. 300° dec.

Anal.—Calc. for $C_{46}H_{48}N_4O_4 \cdot 2HCl \cdot 1.5H_2O$: C, 67.31; H, 6.51; N, 6.83. Found: C, 66.91; H, 6.48; N, 6.50.

3,3'-Diphenyl-2,2'-[azoxydi-(m-phenylene)]-diacrylic Acid, Bis(3-tropanyl) Ester Dihydrochloride, 1.5 Hydrate (VI)—A solution of 3-tropanyl 2-*m*-nitrophenyl-3-phenylacrylate (III, 12.9 g.) in methanol (150 ml.) was hydrogenated over platinum on carbon. The reaction mixture was warmed to dissolve the formed solid, and the solution was filtered. Excess HCl was bubbled into the filtrate, and the solvent was removed. The residue was crystallized from isopropanol–methanol to yield 8.4 g. (67%) of a light-yellow solid, m.p. 283.5–284.5° dec.

Anal.—Calc. for $C_{46}H_{48}N_4O_5 \cdot 2HCl \cdot 1.5H_2O$: C, 66.01; H, 6.38; N, 6.70; Cl, 8.47. Found: C, 65.85; H, 6.51; N, 6.71; Cl, 8.37.

3,3'-Diphenyl-2,2'-[azoxydi-(p-phenylene)]-diacrylic Acid, Bis(3-tropanyl) Ester (XIII)—This was prepared from 3-tropanyl 2-(*p*-nitrophenyl)-3-phenylacrylate (I, 10 g.) by the procedure described for VI. The yellow base (23%) melted at 251–251.5°.

Anal.—Calc. for $C_{46}H_{48}N_4O_5$: C, 74.98; H, 6.57; N, 7.60. Found: C, 74.89; H, 6.59; N, 7.53.

3-Phenyl-2-(p-phenylazophenyl)-acrylic Acid, 3-Tropanyl Ester Hydrochloride (XIV)—This was made by Anspon's method (12). Nitrosobenzene (1.7 g., 0.016 mole) was added to 3-tropanyl 2-(*p*-aminophenyl)-3-phenylacrylate hydrochloride (II, 6.5 g., 0.016 mole) in 39 ml. of cooled glacial acetic acid. The resulting solution was allowed to stand for 16 hr. at room temperature. The product was collected and crystallized from ethanol to give an orange solid (39% yield), m.p. 265.5–267° dec.

Anal.—Calc. for $C_{29}H_{29}N_3O_2 \cdot HCl$: C, 71.37; H, 6.20; N, 8.61; Cl, 7.27. Found: C, 71.38; H, 6.30; N, 8.64; Cl, 7.21.

3-Phenyl-2-(m-phenylazophenyl)-acrylic Acid, 3-Tropanyl Ester Hydrochloride (XV)—This was prepared from 3-tropanyl 2-*m*-aminophenyl-3-phenylacrylate (IV, 6.5 g., 0.016 mole) and nitrosobenzene (1.7 g., 0.016 mole) in glacial acetic acid (39 ml.) by the procedure described for XIV. It was recrystallized twice from isopropanol to give a red-brown solid (59%), m.p. 202–202.5°.

Anal.—Calc. for $C_{29}H_{29}N_3O_2 \cdot HCl$: C, 71.37; H, 6.20; N, 8.61; Cl, 7.27. Found: C, 71.27; H, 6.24; N, 8.39; Cl, 7.30.

3-Phenyl-2-(p-hydroxy-m-phenylazophenyl)-acrylic Acid, 3-Tropanyl Ester, 0.25 Hydrate (XVI)—Benzenediazonium chloride (0.02 mole) was added in six portions to a cooled solution of 3-tropanyl 2-(*p*-hydroxyphenyl)-3-phenylacrylate (7.36 g., 0.02 mole) and 10% NaOH solution (16 ml.) in dimethylformamide (100 ml.). The mixture was stirred for 30 min. and the product was filtered. It was dissolved in tetrahydrofuran, converted to the hydrochloride salt, dissolved in dimethylformamide, neutralized with NaOH solution, and extracted into chloroform. It was recrystallized from methanol to give a red-orange solid (28%), m.p. 188.5–189.5°.

Anal.—Calc. for $C_{29}H_{29}N_3O_3 \cdot 0.25H_2O$: C, 73.78; H, 6.30; N, 8.90. Found: C, 73.85; H, 6.37; N, 8.31.

Pharmacological Methods—The drugs were administered orally by stomach tube in all cases.

Minimum Lethal Dose and Observation of Overt Effects—Groups of three mice were administered various doses of the test drug. Observations for behavioral changes, impairment of reflexes, mydriasis, and lethality were made. The lowest dose causing death was defined as the MLD. Measurements of pupil diameter served to indicate possible anticholinergic activity.

Spasmolytic Activity—A modification of the Janssen *et al.* (14) method was used. Potency was expressed as the dose that reduced the 5-hr. fecal pellet count in mice by 50% as compared to a control group (ED_{50}).

RESULTS

The pharmacological results are shown in Table II. All the compounds were active. In the series with substituents in the 3-position, the 3-aminoester (IV) was six to seven times more potent in the spasmolytic test than the 3-azoester (V), the azoxyester (VI), and the 3-azophenyl ester (XV). Likewise the 4-amino ester (II) was 11–17 times more potent than the 4-nitroester (I), the 4-azoxyester (XIII), the 4-azoester (XII), and the 4-azophenyl ester (XIV).

These data may be interpreted in two ways. The potential aminoester intermediates may be less active *per se* than the amino compounds, or they may appear less active because of the 5-hr. time limit of the spasmolytic test used. In either case, these potential intermediates of the potent aminoesters do not appear to be satisfactory prodrugs.

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ACKNOWLEDGMENTS AND ADDRESSES

Received November 6, 1970, from the *Research and Development Division, Smith Kline & French Laboratories, Philadelphia, PA 19101*
Accepted for publication March 1, 1971.
The authors thank Miss Margarett Carroll and her staff for the microanalysis, Mr. Richard Warren for the UV data, and Mr. John Zarembo for the mass spectral data.

Estimation of Diffusion Coefficients and Molecular Weights of Interacting Colloids from Dissolution-Rate Data

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Abstract □ Based on dissolution-rate theory, diffusion coefficients for the salicylamide–micelle species were calculated from dissolution and solubility data obtained in micellar solutions of sodium taurodeoxycholate and sodium taurocholate at 20°. The calculated values were in good agreement with those determined experimentally using a diffusion cell and silver membrane. By assuming that the solubilized drug does not interfere with the organization of the micelle, the Stokes–Einstein equation was employed to estimate the molecular weight of the micelle. Excellent agreement was found between values calculated from dissolution data and those determined directly by ultracentrifugation or light scattering.

Keyphrases □ Diffusion coefficients, salicylamide–micelle species—calculated from dissolution, solubility data □ Molecular weight determinations, micellar—sodium taurodeoxycholate and sodium taurocholate with salicylamide □ Bile salt solutions—diffusion coefficients, molecular weight estimations, from dissolution-rate data

Numerous methods have been proposed to measure diffusion coefficients of colloids (1–5). These methods often involve rather complex experimental procedures, and much time may be required before experimental results can be obtained.

The most common method of determining diffusion coefficients of colloids has been the use of a diffusion cell and some means to separate the drug solution from the solvent "sink." Desai *et al.* (3) utilized a sintered-glass disk to separate the two solutions. An improvement on the glass disk was reported by Singh *et al.* (4), who used a filter made of glass reinforced with epoxy. Recently, Goldberg and Higuchi (5) successfully measured diffusion coefficients utilizing a silver membrane filter. These workers found that the silver filter produced good data precision and resulted in a fairly rapid method of determining diffusion coefficients.

A number of different investigators (4, 6) confirmed that dissolution from a rotating disk conforms to the theoretical equation presented by Levich (7) for that system. Since the dependence of the dissolution rate on the diffusion coefficient is known for the rotating-disk system, one should be able to use a reversal of the usual procedure—that is, measure dissolution rate of a drug in colloidal and aqueous systems and then calculate the diffusion coefficient of the drug in the system utilizing the Levich equation. This approach could lead to an improvement over the usual methods to determine diffusion coefficients because dissolution-rate measurements are rapid and reproducible. Moreover other factors such as changes in the membrane or cell constant or the incomplete washing out of the diffusion cell would not be problems in the dissolution-rate determination.

THEORY

Levich (7) proposed a convective diffusion theory for the rate of mass transport to or from the face of a rotating disk. The equation for the dissolution rate of a solid in a medium containing a colloidal solubilizing agent according to the rotating disk theory is (4):

$$DR = 0.621\gamma^{-1/6}\omega^{1/2}(D_{\text{eff.}})^{2/3}C_T \qquad \text{(Eq. 1)}$$

where DR is the dissolution rate per unit area of dissolving solid, C_T is the total solubility of the drug in the dissolution medium, γ is the viscosity of the medium, ω is the angular velocity of the rotating disk, and $D_{\text{eff.}}$ is the effective diffusion coefficient. The