Surface character and membranolytic activity of rutile and anatase: two titanium dioxide polymorphs

R P NOLAN, A M LANGER, I WEISMAN, G B HERSON

From the Environmental Sciences Laboratory, Mount Sinai School of Medicine, New York, NY 10029, USA

ABSTRACT Biological studies of two titanium dioxide polymorphs, rutile and anatase, have produced conflicting results. Generally, the in vivo and in vitro methods used to evaluate pneumoconiotic dusts have shown the polymorphs to be inert, but occasionally both minerals have been reported to produce effects consistent with biologically active minerals. Many of these reports failed to specify which polymorph was used experimentally. While this limited the value of the data, the problem was further compounded by the variation in the surface properties of each polymorph depending on whether the specimen was a naturally occurring mineral or was made synthetically. Five naturally occurring and 11 synthetically produced titanium dioxide specimens were studied. The physical characterisation of each specimen entailed the determination of the polymorph type(s) by continuous scan x ray diffraction and the size distribution by transmission electron microscopy. The ability of each specimen to lyse erythrocytes was determined and compared with quartz. Only two, both synthetic rutiles, were found to be active. The hydrogen bonding ability of the surfaces of these rutiles were compared with inert rutile and quartz. The binding properties of the active rutile have been found to be consistent with those properties associated with biologically active quartz. The surface properties of rutile are the determinants of its activity. Because natural and synthetic rutiles possess different surface properties, they display different activities.

The occurrence of interstitial fibrosis, bronchiole-alveolar adenomas and squamous cell carcinomas in rats exposed to high concentrations of synthetic rutile by inhalation has recently been reported.^{1,2} Previously, low biological activity was attributed to the two titanium dioxide polymorphs, rutile and anatase. To emphasize how inert rutile and anatase have been considered, these materials were often used as control or "sham" dusts against which the activity of other mineral particulates were compared.^{3,4} In support of these observations, a few studies of workers engaged in the synthesis of titanium dioxide have failed to show health effects attributed specifically to the inhalation of titanium dioxide dust.^{5,6} Although both titanium dioxide polymorphs have occasionally been found to have in vitro properties consistent with a biologically active particulate,^{7,8} they are today generally considered inert.

Both rutile and anatase are important materials in several consumer products such as pigment in paints, food items and cosmetics.⁹ Titanium dioxide production in the United States for the year 1985 exceeded 800 000 tons (table 1). Virtually all of the synthetically

produced titanium dioxide is in the respirable size range. The widespread use of these materials and their

Table 1 Production of synthetic titanium dioxide in the United States 1985

Producer	Location	Production in tons/year	
		Sulphate*	Chloride*
Dupont de Nemours	Antioch, CA	—	35 000
	DeLisle, MS	—	150 000
	Edge Moor, DE	—	110 000
	New Johnsonville, TN	—	228 000
Kenira Oy (Finnish)	Savannah, GA	66 000	—
Kerr-McGee	Hamilton, MS	—	64 000
SCM Corporation	Ashtabula, OH	—	42 000
	Baltimore, MD	66 000	43 000

*Sulphate process produces anatase; chloride process produces rutile.

NL Industries no longer operate United States plants. Their St Louis, MO, and Sayerville, NJ, plants were closed in 1980. Most sulphate plants closed because of stringent EPA pollution codes.

Foreign produced titanium dioxide now constitutes about 20% of United States consumption: sources from Canada, Norway, Belgium and West Germany.

Based on data from the *United States Minerals Yearbook*, 1981, chapter on titanium by Lynd and Hough, and on data reported in an article by C Verbonic, *Chemical Business*, August, 1985.

Table 2 *x* Ray diffraction characterisation of titanium dioxide specimens used experimentally

Specimen name	Source	% Rutile	% Anatase	Other phases
Natural minerals:				
Rutile	Mozambique, Africa	≥99	0	Tr quartz, clay minerals
Rutile sand	Myrtle Beach, S Carolina	≥98	0	Up to 2% ilmenite, silicates
Rutile	Graves Mountain, Georgia	≥99	0	Tr-1% ilmenite
Rutile	White Mountain, California	≥97	Tr-1	Tr-2% silicates, clay minerals
Anatase	Brazil	Tr-1	≥99	Tr-1% silicates, clay minerals
Synthetic titanium dioxide: industrial powders:				
TiO ₂ , No 10082	N Wotherspoon, MSSM, CUNY	100	0	None detected
Rutile	Dupont Haskell Labs	100	0	Tr phase, unidentified
Titanic oxide	Fisher Scientific	0	100	None detected
Titanium (IV) oxide	Aldrich Chemical	~5	~95	None detected
Polymorphs used in pneumoconiosis laboratories:				
Rutile	PRU No 159, Wales, UK	≥99	Tr-1	None detected
Rutile	IOM-1, Scotland	≥99	Tr-1	None detected
Rutile	IOM-2, Scotland	100	0	None detected
Titanox AWO	R J Schnitzer	Tr-1	≥99	None detected
Anatase specimens: Smithsonian Institution:				
Calcined anatase	K Towe, Smithsonian	~2	~98	None detected
Milled anatase	K Towe, Smithsonian	0	100	None detected
TiO ₂ × H ₂ O	K Towe, Smithsonian	0	100	None detected

The clay minerals include the alteration product leucoxene; the silicate most frequently encountered appears to be a feldspar phase.

PRU = Pneumoconiosis Research Unit of the British MRC.

IOM = Institute of Occupational Medicine, Edinburgh.

MSSM = Mount Sinai School of Medicine.

CUNY = City University of New York.

ubiquitous presence in the environment raises several important issues: What is the biological activity which may be attributed to the titanium dioxide polymorphs, in *all the forms in which it can exist*, and, were the results of the most recent inhalation study valid?¹

The interstitial fibrosis and lung tumours caused by synthetic rutile after inhalation¹ cannot be readily compared with previous reports for the following reasons: (1) the specific titanium dioxide polymorph used experimentally in these studies was often not properly identified¹; (2) no data on particle size distribution were given for the materials used¹⁰; and (3) important surface characteristics of the particle such as the ability to bind specific polymers or the biological activity in one of the *in vitro* assays commonly used in pneumoconiosis research for the assessment of the toxicity of inorganic matter were not determined.¹¹ It would also have been useful if the source of the material had been reported. The activity of minerals in some experiments often reflects the presence or absence of specific trace metals—for example iron—which lessens the activity of other oxide minerals such as quartz.^{12, 13}

The only identification frequently given for materials tested is titanium dioxide.¹ This level of characterisation, however, is incomplete or, at worst, misleading. As examples, Aldrich Chemical Company's titanium oxide-anatase is a mixture of anatase and rutile, Fisher Scientific Company's titanic oxide is pure anatase, and the British MRC Pneumoconiosis Research Units' "respirable TiO₂" used as a control

dust is rutile with a trace of anatase (table 2). The conflicting reports concerning the biological activity of titanium dioxide may thus be impossible to interpret in part related to inadequate characterisation of the mineral.

Some important mineralogical differences among the titanium dioxide polymorphs, which facilitates interpretation of the data presented in this paper, are described in appendix A.

Materials and methods

Sixteen specimens of rutile and anatase were studied experimentally:

NATURALLY OCCURRING TITANIUM DIOXIDE

Minerals rutile, Muiane Altoligoha, Mozambique, Africa—Obtained as two single crystals, together weighing approximately 4 g, from the Department of Mineral Sciences, Catalogue No 27404, American Museum of Natural History, Central Park West, 79th Street, New York, NY 10024.

Rutile sand, Myrtle Beach, South Carolina—Obtained as a coarse powder from Ward's Natural Science Establishment, Inc, Rochester, NY.

Rutile, Graves Mountain, Georgia—Obtained as a large euhedral crystal weighing about 25 g from Ward's Natural Science Establishment, Inc, Rochester, NY.

Rutile, Champion Mine, White Mountains, Mono County, California—Obtained as a large euhedral

crystal weighing about 25 g from Ward's Natural Science Establishment, Inc, Rochester, NY.

Anatase, Minas Gerais, Brazil—Obtained as a collection of pellets from Ward's Natural Science Establishment, Inc, Rochester, NY.

These five specimens were ground dry, by hand, with a carborundum mortar and pestle until the particle size was fine enough to pass through a 45 μm mesh screen.

SYNTHETIC TITANIUM DIOXIDE PREPARATIONS
The following specimens were obtained as fine industrial powders:

Titanium dioxide No 10082—Obtained from N Wotherspoon, Mount Sinai School of Medicine (reportedly a rutile made by Dupont).

Titanium dioxide, rutile—Central Research and Development Department, EI Dupont de Nemours & Co, Inc, Haskell Laboratory for Toxicology & Industrial Medicine, Haskell No 11 564, PO Box 50, Elkton Road, Newark, Delaware 19714.

Titanic oxide anhydrous—Fisher Scientific Company, Fairlawn, NJ (chemical reagent grade).

Titanium (IV) oxide anatase—Aldrich Chemical Company, Inc, Milwaukee, WI (chemical reagent grade).

TITANIUM DIOXIDE SPECIMENS USED IN PNEUMOCONIOSIS LABORATORIES AS CONTROLS
The following specimens, obtained from pneumoconiosis laboratories, have been used as inert control dusts experimentally.

Respirable TiO_2 —PRU No 159 (Hexhlet collected) obtained from R Davies, MCR Pneumoconiosis Unit, Llandough Hospital, Penarth, S Glamorgan UK.

Titanium dioxide—Obtained as two separate specimens called IOM-1 and IOM-2 from K Donaldson, Institute of Occupational Medicine, Roxburgh Place, Edinburgh, UK.

Titanox AWO—Obtained from RJ Schnitzer, Mount Sinai School of Medicine.

ANATASE SPECIMENS

The following three specimens were obtained from K Towe, Smithsonian Institution, Washington, DC:

Calcined anatase—Origin through industrial processing; synthetic.

Milled anatase—Origin through industrial processing; synthetic.

Precipitated $\text{TiO}_2 \cdot x\text{H}_2\text{O}$ —Origin through industrial processing; synthetic.

The following quartz specimens were obtained as fine industrial powders from Pennsylvania Glass and Sand Company and were used as positive controls in the haemolytic test systems.

Min-U-Sil 5 and Min-U-Sil 15—Both specimens were obtained as fine industrial quartz powders. The 5 and 15 denotes that 98% of the particles are less than 5 and 15 μm in size, respectively. The 1 and 2 μm Stokes' diameter of each specimen was separated by sedimentation techniques.¹⁴ Min-U-Sil 15 was used as a positive control.¹⁵

Binding studies, for determination of the hydrogen bonding character of the mineral surface, were carried out with:

2-poly(vinylpyridine-N-oxide) obtained from Polyscience, Inc, Warrington, PA. The weight average molecular weight of the 2-PVPNO polymer, as determined by light scattering, was $\sim 276\,000$ (R P Nolan and A M Langer, unpublished data).

CHARACTERISATION OF SPECIMENS

The examination of these specimens by polarised light microscopy and continuous scan x ray diffraction was carried out to determine the presence or absence of crystalline impurities, ascertain gross size distribution, and identify specific polymorphic phase.

POLARISED LIGHT MICROSCOPY

About 0.1 mg of powder was withdrawn from each specimen and placed on a glass slide. The powder was covered with an immersion oil ($n = 1.52$) and dispersed by sliding another glass slide across the preparation surface. Virtually every specimen smeared to yield a uniform homogeneous distribution. The natural specimens produced a yellow brown opaque suspension in natural light whereas the synthetic specimens produced an opaque white suspension. The smear was further "wetted" with an additional drop of immersion oil and then a cover slip placed on its surface. The preparations were examined by polarised light microscopy, both in plane polarised light and between crossed Nichols, at magnification ranging from $35\times$ to $500\times$ magnification.

The natural minerals are composed of large sized, yellow brown fragments. Some particles are 100 μm in greatest dimension but the numerically preponderant size is about 3–5 μm . Single rutile particles show twin plane lamellae, as well as some mineral intergrowths. These latter impurities are clearly evident when specimens are viewed between crossed Nicols. Quartz, other silicates—for example, micas and clays, and brown opaques (limonite minerals?)—are observable in several specimens and are not restricted to any single polymorphic species.

By contrast, the synthetic preparations are all fine grained with particles $< 1\,\mu\text{m}$ in size, and with no visible mineral impurity. The characteristic features of the synthetic materials are their small and uniform

Table 3 Size distribution of titanium dioxide and quartz specimens

Percentage within each size class								
<i>Natural titanium dioxide minerals</i>								
<i>Specimen</i>	<i>Phase*</i>	<i>No†</i>	<i>< 0.50 µm</i>	<i>0.51–1.00 µm</i>	<i>1.01–2.00 µm</i>	<i>2.01–5.00 µm</i>	<i>5.01–10.00 µm</i>	<i>> 10.1 µm</i>
Mozambique	R	597	51.6	16.2	20.8	8.6	2.8	0.0
Myrtle Beach	R	767	48.9	21.6	17.9	9.0	2.1	0.5
Graves Mountain	R	783	41.4	26.0	22.0	8.4	1.8	0.4
White Mountain	R	735	60.7	22.9	11.5	4.9	0.0	0.0
Brazil	A	783	61.4	23.0	12.0	3.2	0.4	0.0
<i>Industrial powders (synthetics)</i>								
<i>Specimen</i>	<i>Phase</i>	<i>No</i>	<i>< 0.25 µm</i>	<i>0.26–0.50 µm</i>	<i>0.51–1.00 µm</i>	<i>> 1.01 µm</i>		
No 10082	R	309	66.4	33.3	0.3	0.0		
Dupont	R	376	62.2	37.3	0.5	0.0		
Fisher	A	376	88.1	9.8	2.2	0.0		
Aldrich	A	332	86.1	13.0	0.9	0.0		
<i>Titanium dioxide used in pneumoconiosis laboratories as control dusts</i>								
<i>Specimen</i>	<i>Phase</i>	<i>No</i>	<i>< 0.25 µm</i>	<i>0.26–0.50 µm</i>	<i>0.51–1.00 µm</i>	<i>> 1.01 µm</i>		
PRU No 159	R	320	48.8	51.2	0.0	0.0		
IOM-1	R	344	46.0	51.7	2.3	0.0		
IOM-2	R	200	43.5	49.5	7.0	0.0		
Titanox AWO	A	219	92.2	7.8	0.0	0.0		
<i>Anatase specimens: Smithsonian Institution</i>								
<i>Specimen</i>	<i>Phase</i>	<i>No</i>	<i>< 0.25 µm</i>	<i>0.26–0.50 µm</i>	<i>0.51–1.00 µm</i>	<i>> 1.01 µm</i>		
Calcined	A	345	76.5	22.6	0.9	0.0		
Milled	A	376	96.8	3.2	0.0	0.0		
Precipitated	A	—	100.0	—	—	—		
<i>Min-U-Sil (quartz) controls</i>								
<i>Specimen</i>	<i>Phase</i>	<i>No</i>	<i>< 0.50 µm</i>	<i>0.51–1.00 µm</i>	<i>1.01–2.00 µm</i>	<i>2.01–5.00 µm</i>	<i>> 5.01 µm</i>	
MUS 5 1 µm	Q	380	17.6	26.3	33.4	21.9	0.8	
MUS 5 2 µm	Q	387	18.1	19.4	13.4	30.3	18.8	
MUS 15 1 µm	Q	443	24.6	32.1	26.6	16.2	0.5	
MUS 15 2 µm	Q	307	12.7	14.7	7.5	43.6	21.5	

*R or A denotes rutile or anatase, respectively.

†Number of particles counted.

particle sizes, specimen purity, and opacity to light when viewed under plane polarised conditions.

Continuous scan x ray diffraction

Two hundred milligrams of powder were withdrawn from each specimen, tamped into a 1×2 cm area in an aluminium specimen holder, and subjected to continuous scan x ray diffraction analysis. The experimental conditions are identical with those used for the analysis of silica specimens.¹⁵ The x ray diffraction tracings were converted to d-spacings and these were compared with standard diffraction data for rutile (ASTM 21-1276), anatase (ASTM 21-1272), and other minerals. The results are given in table 2.

SIZE DISTRIBUTION MEASUREMENTS

Each titanium dioxide specimen and the quartz 1 and 2 μm Stokes's diameter specimens fractionated by sedimentation from Min-U-Sil 5 and Min-U-Sil 15 were photographed by transmission electron microscopy at $2000 \times$ direct magnification, then each plate was enlarged $2.5 \times$ at printing to yield a final magnification of $5000 \times$. The longest dimensional

aspect was measured as particle diameter. The results are, therefore, skewed toward larger particle size values. The particle size distribution of the titanium dioxide specimens studied and the five quartz specimens used as controls are given in table 3 and reference 15.

HAEMOLYTIC MODEL

The ability of each of the titanium dioxide and quartz specimens to alter the permeability of human erythrocytes was determined quantitatively. The erythrocytes were obtained from peripheral blood (50 ml) taken by venepuncture in a plastic syringe containing 15 U/ml preservative free heparin. Ficoll-Hypaque gradient centrifugation was used to separate the peripheral blood lymphocytes and plasma proteins from the erythrocytes. Fifty millilitres of blood was diluted with an equal volume of saline, overlaid on to a 25 ml Ficoll-Hypaque gradient, and centrifuged at $F \sim 850$ g at 20°C for 45 minutes. The lymphocytes and plasma proteins were removed and the erythrocytes were stored at 4°C and used within the week.

Fig 1 Comparison of membranolytic activity of two fine particle size quartz specimens and two synthetic rutile specimens.

Before the stored erythrocytes were used, about 5 ml of packed human erythrocytes were diluted to 50 ml with pH 7.4 veronal buffered saline (VBS, 2.4 mM sodium barbital and 145 mM sodium chloride) in a plastic centrifuge tube and spun at $F \sim 800$ g, at room temperature, for 30 minutes. The erythrocyte wash was repeated twice. Using optical spectroscopy, the washed human erythrocytes were made into a standard suspension which contained about 3.6×10^8 cells/ml.¹⁵

A single stock suspension of each specimen was also made in VBS. To disperse the suspensions, each was sonicated using a sonifier cell disrupter (heat systems—Ultrasonics Inc) at 50 W power for several minutes until the suspension was visibly homogeneous. Each of the stock mineral suspensions were diluted to four concentrations (mg/ml) in duplicate. Each of the four different dilutions were adjusted to 4 ml, and then 4 ml of the standard erythrocyte suspension was added. The final volume at each concentration was therefore 8 ml, and the erythrocyte concentration was 1.8×10^8 cells/ml. The powder and erythrocytes were incubated at 37°C for 120 minutes and shaken continuously with a gyratory motion. The plotted relation of mg/ml of mineral against haemolysis is generally linear up to about 40% haemolysis (all measurements reported in this

Fig 2 Hydrogen bonding ability of two fine particle size quartz specimens as compared with four titanium dioxide specimens.

paper fell under this value). The duplicate determinations were averaged and the values are shown with their variation. Linear regression analysis was made for each set of eight points and the equation of the line calculated and used to compute the HC50, the concentration of powder (in mg/ml) required to lyse half of the erythrocytes in the suspension.

DETERMINATION OF

2-POLY(VINYLPYRIDINE-N-OXIDE) BINDING

The amount of 2-PVPNO that bound to selected titanium dioxide and quartz specimens was determined quantitatively. Various amounts of the mineral powders were weighed out into glass centrifuge tubes and to each was added 30 ml of 50 µg/ml 2-PVPNO in distilled water. The tubes were allowed to stand at room temperature for 30 minutes. The tubes were then centrifuged at $F \sim 8000$ g, at room temperature, for 40 minutes, which pelleted the powder at the bottom of the tubes. The amount of polymer able to bind to each concentration of powder is thereby removed from the supernatant. The 2-PVPNO polymer has an absorbance peak in the ultraviolet at 260 nm, and a molar absorptivity, E , of 5631, which was calculated using the molecular weight of the monomer (MW 121.1). The absorbance

Table 4 Membranolytic activity of the naturally occurring titanium dioxide polymorphs: rutile and anatase

TiO_2 (mg/ml)	Anatase Brazil (%H)	Rutile sand S Carolina (%H)	Rutile California (%H)	Rutile Georgia (%H)	Rutile Africa (%H)	Min-U-Sil 15	
						(mg/ml)	(%H)
0.625	1.3 ± 0.1	1.0 ± 0.0	1.2 ± 0.0	1.1 ± 0.0	1.2 ± 0.0	0.375	5.2 ± 0.0
1.250	1.8 ± 0.0	1.1 ± 0.0	1.8 ± 0.2	1.2 ± 0.1	1.5 ± 0.0	0.750	11.4 ± 0.0
1.875	2.2 ± 0.0	1.3 ± 0.1	2.5 ± 0.4	1.5 ± 0.0	1.8 ± 0.1	1.125	16.7 ± 0.0
2.500	2.5 ± 0.3	1.5 ± 0.0	3.7 ± 0.2	1.9 ± 0.0	1.8 ± 0.1	1.500	21.6 ± 0.1

%H = Per cent haemolysis.

Table 5 Membranolytic activity of titanium dioxide specimens obtained as fine industrial powders (synthetics)

Amount TiO ₂ (mg/ml)	TiO ₂ -Haskell Laboratory (%H)	TiO ₂ , No 10082 (%H)	Amount TiO ₂ (mg/ml)	Calcined anatase (%H)	Milled anatase (%H)	TiO ₂ × H ₂ O anatase (%H)
0.313	5.1 ± 0.3	6.4 ± 0.3	0.50	1.2 ± 0.1	1.2 ± 0.1	1.5 ± 0.1
0.625	14.3 ± 0.8	16.4 ± 2.2	1.00	1.2 ± 0.1	1.4 ± 0.1	2.0 ± 0.1
0.938	27.6 ± 3.1	30.7 ± 1.2	1.50	1.1 ± 0.1	1.5 ± 0.1	2.7 ± 0.0
1.250	38.0 ± 4.5	37.5 ± 2.2	2.00	1.0 ± 0.1	1.7 ± 0.1	3.4 ± 0.3

Amount TiO ₂ (mg/ml)	Anatase titanox (%H)	Anatase Aldrich (%H)	Anatase titanic oxide Fisher (%H)	Amount Min-U-Sil 15 (mg/ml)	(%H)
0.625	3.0 ± 0.1	2.1 ± 0.2	1.9 ± 0.1	0.375	7.7 ± 0.2
1.250	3.8 ± 0.3	2.9 ± 0.1	2.3 ± 0.1	0.750	15.3 ± 0.4
1.875	4.8 ± 0.1	4.0 ± 0.1	4.2 ± 0.1	1.125	21.8 ± 0.4
2.500	5.9 ± 0.0	5.3 ± 0.1	4.4 ± 0.2	1.500	27.6 ± 0.6

%H = Per cent haemolysis.

Fig 3 Comparison of membranolytic activity of six synthetic titanium dioxide specimens and quartz.

Fig 5 Ability of four synthetic rutiles to hydrogen bond with the polymer 2-PVPNO.

Fig 4 Comparison of a membranolytically active Haskell rutile (Dupont) with two fine particle size quartz specimens and two titanium dioxide specimens used in pneumoconiosis research as inert controls.

Fig 6 Inhibition of titanium dioxide No 10082 membranolytic activity with 2-PVPNO.

of the supernatant was then measured at 260 nm and the concentration of 2-PVPNO "lost" from the supernatant was assumed to be bound to the powder surface. The technique has been used for silica preparations.¹⁵ The data were plotted graphically as mineral powder ($\mu\text{g/ml}$) v 2-PVPNO ($\mu\text{g/ml}$) (figs 2 and 5).

POSITIVE CONTROLS

Unfractionated Min-U-Sil 15 was included in each experiment as a positive control. Differences in its HC50 value indicate the extent of difference among the erythrocyte suspensions which may exist due to donor, for example.

Experimental results

MEMBRANOLYTIC ACTIVITY OF THE TITANIUM DIOXIDE SPECIMENS

Of the five naturally occurring rutile and anatase specimens studied, none was found to have significant membranolytic activity (table 4). Of the 11 titanium dioxide specimens obtained as fine industrial powders, only two were found to be membranolytically active (table 5, figs 1, 3). These, the Haskell Laboratory rutile and TiO_2 No 10082, were severely haemolytic. The membranolytic activity of Haskell (Dupont) rutile was compared with four fine particle size quartz specimens and two titanium dioxide specimens used as inert controls in experimental pneumoconiosis (fig 4). The precipitated $\text{TiO}_2 \cdot x\text{H}_2\text{O}$, when suspended in VBS, was found to lower the pH to 3 but was adjusted back to pH 7.4 with 1N sodium hydroxide.

COMPARISON OF THE BONDING ABILITY OF THE TITANIUM DIOXIDE SPECIMENS AND QUARTZ WITH 2-PVPNO

The biological activity of quartz, a potent fibrogenic mineral, is related to the ability of that mineral to bind 2-PVPNO¹⁶ The current binding studies were carried out to determine if the different membrane behaviour observed for the titanium dioxide specimens could be distinguished on the basis of their differential 2-PVPNO binding abilities. If a titanium dioxide specimen could bind the polymer would it act as an inhibitor of membrane activity, as happens with quartz?

The ability of two membranolytically active rutile specimens to bind 2-PVPNO was quantitatively compared with two fine sized quartz specimens and several of the non-membrane active titanium dioxide specimens. The Haskell rutile (Dupont) and TiO_2 No 10082 have hydrogen bonding ability comparable with the 1 and $2\mu\text{m}$ Stokes's diameter fractions of Min-U-Sil 15 (figs 2 and 5). The two membrano-

lytically active TiO_2 specimens are of a smaller particle size and thus greater surface area than the two fine particle size quartz specimens.

The titanic oxide from Fisher did not have the appropriate surface chemistry to hydrogen bond the polymer whereas the rutile from White Mountain, California, bound relatively high amounts of 2-PVPNO. The amount of 2-PVPNO bound by the California rutile is even more significant considering it is of a much more coarse particle size than the Fisher specimen and therefore has much less surface area (table 3 and fig 2). The concentration of the TiO_2 s used in the experiment (fig 1), 5–40 mg/ml, was on the plateau of the adsorption isotherm.¹⁷ The polymer binding experiment was repeated at lower concentrations of TiO_2 so that an increase in binding could be shown as a function of TiO_2 concentration (fig 5). The titanium (IV) oxide and rutile IOM-1 bound significantly less 2-PVPNO than the two membranolytically active TiO_2 specimens.

The TiO_2 No 10082 was found to be the most haemolytic of all the titanium dioxide specimens studied. The concentration of TiO_2 No 10082 was held constant at 2.25 mg/ml and the amount of 2-PVPNO per tube was varied from 0–25 $\mu\text{g/ml}$. The TiO_2 /2-PVPNO tubes were allowed to stand for 30 minutes at room temperature and then challenged with standard erythrocyte suspension as described in the materials and methods section. Almost 88% of the membranolytic activity was inhibited at the highest concentration of 2-PVPNO (fig 6). The action of polymer binding on the titanium dioxide specimens and its subsequent antagonism of membrane activity parallels the effect of 2-PVPNO on quartz.

Discussion and conclusions

The biological potential of the titanium dioxide polymorphs, rutile and anatase, is incompletely understood. Although both these polymorphs commonly exist in the environment, experimental studies regarding their activities have rarely specified which specific mineral was studied.

The surface properties of the two polymorphs vary depending on whether they are naturally occurring or synthetically made. The surface properties of synthetic rutile vary greatly, apparently dependent on the conditions of the synthetic process. Therefore, synthesis may well determine membrane activity. These differences in surface properties make it more difficult to define the biological potential of synthetic rutile and anatase because these surface properties must be measured.

The naturally occurring specimens of rutile and anatase used in this study contained a few large, coloured particles: the Brazilian anatase fragments

Fig 7 Photomicrograph of four titanium dioxide specimens: (a) rutile, Africa; (b) rutile, synthetic, Haskell (Dupont); (c) anatase, Brazil; and (d) rutile, South Carolina. Black bar represents 40 μm .

were up to 100 μm in size and were cream coloured; all the other natural rutiles also contained large, brownish fragments. These colours suggest the presence of trace metal and mineral impurities. Both these factors have been linked to reduced membrane

activity in the silica polymorphs.^{12 13}

Synthetic rutile or anatase form white homogeneously fine powders. Several of the synthetic preparations were mixtures of both polymorphs (table 2). The synthetic polymorphs differ in

Table 6 The HC50 of 1 μ m Stokes's diameter Min-U-Sil 15 and Haskell (Dupont) rutile calculated in mg/ml, particle number/ml, and surface area/ml

	mg/ml	Particles/ml	Surface area (μm^2)/ml
1 μ m Stokes's diameter Min-U-Sil 15 (n = 4)*	0.3 $\pm$ 0.0	1.1 $\times 10^8$	6.9 $\times 10^8$
Haskell (Dupont) Rutile (n = 8)	2.1 $\pm$ 0.4	6.1 $\times 10^{10}$	1.5 $\times 10^{10}$

*n = Number of determinations.

composition, colour, and particle size as compared with the natural minerals. Virtually every particle within the synthetic specimens is less than 1.0 μ m in size (see table 3). It should be noted that the synthetic materials also differ from the naturally occurring materials in particle shape, tending to be oval to rounded, with fewer straight edges (fig 7).

Only two of the 16 specimens tested were found to be membranolytically active in a human erythrocyte model. All the naturally occurring rutiles, and all the anatase specimens, both the natural mineral and its synthetic specimens, were found to be inactive (tables 4 and 5). Of the five synthetic rutiles studied, only the Haskell rutile (Dupont) and TiO₂ No 10082, were found to be active (figs 3 and 4). Other investigators have observed synthetic anatase to be active and rutile to be inert.⁸

The membranolytic activity of Haskell rutile was compared with four fine particle size quartz specimens. On the basis of mass, the quartz specimens were *all* more active than Haskell (Dupont) rutile (figs 1, 3, and 4). The difference in activity between the rutile and quartz specimens is even greater than the mass data implies. For example, the 1 μ m Stokes's diameter Min-U-Sil 15 was seven times more membranolytic than the Haskell rutile on the basis of mass. Assuming the Haskell rutile and 1 μ m Stokes's diameter Min-U-Sil 15 to be 0.2 and 1 μ m cubes respectively the Haskell rutile required 555 times more particles (roughly 22 times more surface area) to

achieve the same membranolytic activity (table 6). Although the Haskell material is membranolytic, it is significantly less active than quartz. Assuming the particles to be cubic, the relation between particle size and the number of particles and surface area per unit mass is shown in table 7.

The ability of several of the titanium dioxide specimens to bind 2-PVPNO was compared with 1 and 2 μ m Stokes's diameter Min-U-Sil 15. The two active rutiles had the appropriate surface chemistry to hydrogen bond the polymer as did the naturally occurring rutile from White Mountain, California (figs 2, 5). The 2-PVPNO was found to inhibit haemolysis by TiO₂ No 10082. The maximum inhibition for No 10082 (fig 6) occurred at about the same concentration of 2-PVPNO as the maximum polymer binding for that TiO₂ at that specific mineral concentration (fig 5). Two other synthetic rutiles and one anatase, all inactive, were unable to bind the polymer. As a point of comparison, experimental data indicate that the biological activity of quartz is related to the mineral's ability to hydrogen bond 2-PVPNO.^{11 16}

The PRU titanium dioxide, the two Institute of Occupational Medicine specimens, and the Haskell specimen have been shown by x ray diffractometry to be rutile. The PRU rutile has been shown to be weakly cytotoxic to macrophages.¹⁸ The in vitro properties of the IOM-1 were consistent with an inert dust.^{19 20} Also, this specimen was weakly inflammatory in vivo.²¹ Electron microscopic studies show them all to be of similar particle size and therefore to have comparable surface area. The surface properties of the active rutile, however, clearly differ from that of the other three specimens. These chemical differences are recognised by the erythrocyte membrane.

The titanium dioxide polymorphs possess a range of biological activities depending on the polymorph type, the chemical functionalities at the particle surface, particle size distribution, and trace metal content. Synthetic titanium dioxide may have

Table 7 Relation between particle size and surface area for cubes of quartz, anatase, and rutile

Particle size (μm)	No of particles/mg			Surface area (μm^2)/particle	Surface area (μm^2)/mg		
	Quartz	Anatase	Rutile		Quartz	Anatase	Rutile
0.2	4.7 $\times 10^{10}$	3.2 $\times 10^{10}$	2.9 $\times 10^{10}$	0.24	1.1 $\times 10^{10}$	7.7 $\times 10^9$	7.0 $\times 10^9$
0.5	3.0 $\times 10^9$	2.1 $\times 10^9$	1.9 $\times 10^9$	1.5	4.5 $\times 10^9$	3.2 $\times 10^9$	2.9 $\times 10^9$
1.0	3.8 $\times 10^8$	2.6 $\times 10^8$	2.4 $\times 10^8$	6.0	2.3 $\times 10^9$	1.6 $\times 10^9$	1.4 $\times 10^9$
2.0	4.7 $\times 10^7$	3.2 $\times 10^7$	2.9 $\times 10^7$	24.0	1.1 $\times 10^9$	7.7 $\times 10^8$	7.0 $\times 10^8$
5.0	3.0 $\times 10^6$	2.1 $\times 10^6$	1.9 $\times 10^6$	150.0	4.5 $\times 10^8$	3.2 $\times 10^8$	2.9 $\times 10^8$
10.0	3.8 $\times 10^5$	2.6 $\times 10^5$	2.4 $\times 10^5$	600.0	2.3 $\times 10^8$	1.6 $\times 10^8$	1.4 $\times 10^8$
20.0	4.7 $\times 10^4$	3.2 $\times 10^4$	2.9 $\times 10^4$	2400.0	1.1 $\times 10^8$	7.7 $\times 10^7$	7.0 $\times 10^7$

Density of quartz $\sim$ 2.656 g/cc No 5-0490 (SPDF).Density of anatase $\sim$ 3.893 g/cc No 21-1272 (SPDF).Density of rutile $\sim$ 4.250 g/cc No 21-1276 (SPDF).

Table 8 Comparison of the physical and chemical properties of the titanium dioxide polymorphs

Property	Titanium dioxide polymorph		
	Rutile	Anatase*	Brookite
x Ray data ASTM SPDF:	21-1276	21-1272	16-617
Crystal system	Tetragonal	Tetragonal	Orthorhombic
Space group	P4 ₂ /m nm (136)	14 ₂ /a md (141)	P cab (61)
Unit cell x axis	4.5933 (4.59)	3.7852 (3.78)	9.1819 (9.14)
y axis			5.4559 (5.44)
z axis	2.9592 (2.96)	9.5139 (9.50)	5.1429 (5.15)
Z cell	2	4	8
Density†	4.250 (4.18-4.25)	3.893 (3.82-3.95)	4.119 (3.87-4.12)
Packing coordination	6:3 HCP	6:3 CCP	6:3
Bulk chemistry:	TiO ₂	TiO ₂	TiO ₂
Trace metals	Cr, V, Fe, Al, Sn, Mn, H ₂ O, Ta, Nb	V, Fe, Al, Sn, Ca	Fe, Mg, many Tr metals reported
Trace minerals	Quartz, ilmenite, biotite, others	Many minerals reported	Many minerals reported
Planes of failure:			
Twinning	e (101) Common v (301) Rare (011) Glide	(112) Rare	(230) Possible
Cleavage	a (100) Moderate m (110) Good s (111) Traces	c (001) Perfect p (011) Perfect	m (120) Poor c (100) Very poor
Parting	Along glide planes		
Fracture	Uneven, subconchoidal	Uneven, subconchoidal	Uneven, subconchoidal
Stability - T, pH	Highest 2 shared edges	Least 3 shared edges	Intermediate 4 shared edges 1 shared face
Colour‡	R, B, Br, V, Y, G	Br, Y, G, B, Bl	R, Br, B, V
Dispersion	Very strong	Strong	Strong, variable
Refractive Index	W 2.605-2.613 E 2.899-2.901 J 0.286-0.296	E 2.488 W 2.501 J 0.073	

*Anatase is also referred to as octahedrite in older mineralogical reports.

†Density appears to vary according to iron content which may occur as a "minor" element rather than as a "trace" element (some %). The density of anatase changes dramatically after heating (d = 4-16).

‡Colours are R = red, B = blue, Br = brown, V = violet, Y = yellow, G = green, Bl = black. Synthetic rutile tends to be white. Titanium in pulmonary tissues tends to be greenish white.

profoundly different surface properties as compared with its natural mineral counterpart. It follows that biological activity, as gauged by membranolytic activity, will differ considerably as well.

Appendix A

NATURALLY OCCURRING TITANIUM DIOXIDE POLYMORPHS

Three titanium dioxide polymorphs have been identified in nature: rutile, anatase, and brookite.²² Brookite is unimportant in terms of human exposure and is included in the data comparison (table 8) only for completeness. Rutile and anatase are formed under a wide range of physicochemical conditions and are present throughout several geological provenances. Although common in nature, industrially used titanium dioxide is mostly the product of chemical synthesis.

CRYSTAL STRUCTURE OF THE TITANIUM DIOXIDE POLYMORPHS

The three crystal structures of TiO₂ are made of titanium octahedrally coordinated with oxygen

atoms. All the polymorphs display a sixfold coordination of oxygen about a titanium atom, with each of the oxygens occupying the corners of a regular octahedron. In turn, every oxygen atom is coordinated with three titanium atoms occupying the approximate corners of an equilateral triangle. This structure has been referred to as a 6:3 coordination and is referred to by crystallographers as the "rutile structure" (table 8).^{23, 24} Though this coordination is constant, the octahedra bond angles and distances differ among the polymorphs so that the sharing of edges of the octahedrons increase from two in rutile to three in brookite to four in anatase. For the two commercially important polymorphs, rutile and anatase, the position of the octahedra in the lattice and the number of octahedra in the unit cell differ (see table 8 and fig 8). Anatase contains four TiO₂ molecules per unit cell while rutile contains two (table 8). As edges and faces of the octahedra are increasingly shared, the titanium distances decrease, setting up cation repulsive forces. This decreases the stability of the mineral structure and is reflected in such basic features as hardness and solubility. Rutile, the most stable, is the hardest and least soluble polymorph.²⁵

Fig 8 Crystal structure of (a) rutile and (b) anatase.

CRYSTAL STRUCTURE AND SURFACES OF TITANIUM DIOXIDE POLYMORPHS

The small variations in the structure of rutile and anatase lead to different cleavage and twin planes along which separation tends to occur. Therefore, one would assume that for anatase and rutile the density of surface titanols and their isoelectric point for anatase and rutile would differ regardless of whether the crystals were similarly grown to a certain size or reduced in size from larger particles (table 8).²⁶

PREPARATION OF SYNTHETIC TITANIUM DIOXIDE FOR PIGMENT

The sulphate and chloride processes are the two used commercially to produce synthetic rutile and anatase. The sulphate process is the older of the two and, though being superseded by the chloride, is still used to make the bulk of the world's TiO_2 pigment product. Here, ilmenite ore (FeTiO_3) is converted to TiOSO_4 after reaction with concentrated sulphuric

acid at 150–180 °C:

This solution is cooled, filtered, and concentrated by evaporation under reduced pressure to crystallise the ferrous sulphate. The crystalline FeSO_4 is separated. The titanium sulphate solution is then hydrolysed by heating to 90 °C to form insoluble titanyl hydroxide:

The titanyl hydroxide precipitate is then separated from the solution by filtration and washed extensively to remove all traces of discolouring elements (Fe, Cr, V, and Mn). The hydrated precipitate is then modified to produce the characteristics desired in a pigment. If the solution is seeded with rutile before hydrolysis then rutile is formed. In the absence of seed crystals the product will be anatase. The modified precipitate is calcined at 800–1000 °C (see fig 9).

The chloride process was commercially developed in the 1950s and requires an enriched titanium dioxide ore. The ilmenite may be beneficiated or natural rutile used. The high grade titanium dioxide ore (70–85% TiO_2) is chlorinated in a fluidised bed reactor with coke at 925–1010 °C:

The volatile titanium tetrachloride is then separated from the unreacted solids and non-volatile chlorides by double distillation. Impurities can alter

Fig 9 Flow diagram of sulphate process.

Fig 10 Flow diagram of chloride process.

the brightness and colour of the pigment. The TiCl_4 is then reacted with O_2 in a flame reaction at 925°C . This reaction produces fine particle TiO_2 and liberates Cl_2 gas, which is recycled (see figure 10):

The low temperature sulphate process may be used to produce either 100% rutile or 100% anatase, whereas the high temperature chloride process cannot be used to produce a 100% anatase product and is generally used to produce rutile.²⁷⁻³⁰

This study was made possible by support from the National Institute of Environmental Health Sciences ES00928, National Institute for Occupational Safety and Health OH 01905, and the Societe Nationale De L'Amiante, Canada. Robert P Nolan wishes to acknowledge support as a fellow of the Stony Wold-Herbert Fund, Inc. Present address of RPN and AML: Center for Polypeptide and Membrane Research, Mount Sinai School of Medicine.

References

- Lee KP, Trochimowicz HJ, Reinhardt CF. Pulmonary response of rats exposed to titanium dioxide (TiO_2) by inhalation for two years. *Toxicol Appl Pharmacol* 1985;79:179-92.
- Lee KP, Trochimowicz HJ, Reinhardt CF. Transmigration of titanium dioxide (TiO_2) particles in rats after inhalation exposure. *Exp Mol Pathol* 1985;42:331-43.
- Ferin J, Oberdorster G. Biological effects and toxicity assessment of titanium dioxides: anatase and rutile. *Am Ind Hyg Assoc J* 1985;46:69-72.
- Richard RJ, White LR, Eik-Nes KB. Biological reactivity of different crystalline forms of titanium dioxide in vitro and in vivo. *Scand J Work Environ Health* 1985;11:317-20.
- Uragado CG, Pinto MRM. An investigation into the health of workers in an ilmenite extracting plant. *Med J Aust* 1972;1:167-9.
- Daum SM, Anderson HA, Lilis R, et al. Pulmonary changes among titanium workers. *Proceedings of the Royal Society of Medicine* 1977;70:31-2.
- Harrington JS, Macnab GM, Miller K, et al. Enhancement of haemolytic activity of asbestos by heat-labile factors in fresh serum. *Med Lav* 1971;62:171-6.
- Zitting A, Skytta E. Biological activity of titanium dioxide. *Int Arch Occup Environ Health* 1979;43:93-7.
- Verbonic C. Titanium dioxide: will its dazzle dim? *Chemical Business* 1985; August: 10-4.
- Leykow W, Gendek E. A spin label study of the effect of asbestos, quartz, and titanium dioxide dust on the bovine erythrocyte membrane. *Br J Ind Med* 1985;42:281-4.
- Beck EG, Bignon J, eds. *In vitro effects of mineral dusts. Third international workshop*. Berlin: Springer-Verlag, 1985.
- Langer AM. Crystal faces and cleavage planes in quartz as templates in biological process. *Q Rev Biophys* 1978;11:543-75.
- Engelbrecht FM, Thiar BF. The effect of small amounts of aluminium, carbon and carborundum on the development of silicosis and asbestosis. *South African Medical Journal* 1972;46:462-4.
- Nolan RP, Langer AM. Quartz and hemolysis: physico-chemical factors controlling membrane activity. In: Wagner W, Merchant J, Rom W, eds. *Health issues related to mining and minerals*. Michigan, Ann Arbor Press, 1983:63-81.
- Nolan RP, Langer AM, Harrington JS, et al. Quartz hemolysis as related to its surface functionalities. *Environ Res* 1981;26:503-20.
- Nash T, Allison AC, Harrington JS. Physico-chemical properties of silica in relationship to its toxicity. *Nature* 1966;210:259-61.
- Adamson AW. *Physical chemistry of surfaces*. New York: Wiley-Interscience, 1982.
- Davies R, Griffiths DM, Johnson NF, et al. The cytotoxicity of kaolin toward macrophages in vitro. *Br J Exp Pathol* 1984;65:453-66.
- Gormley IP, Collings P, Davis JMG, et al. An investigation into the cytotoxicity of respirable dusts from British collieries. *Br J Exp Pathol* 1979;60:526-36.
- Gormley IP, Bolton RE, Brown GM, et al. Some observations on the in vitro cytotoxicity of chrysotile prepared by the wet dispersion process. *Environ Health Perspect* 1983;51:35-9.
- Donaldson K, Bolton RE, Brown D. *Inflammatory cell recruitment as a measure of mineral dust toxicity*. In: Dodgson J, McCallum RI, eds. *Vth International Symposium on Inhaled Particles*. Cambridge, 1985. Oxford: Pergamon Press (in press).
- Deer WA, Howie RA, Zussman J. *Rock-forming minerals. Non-silicates*. Vol 5. London: Longman 1972:34-47.
- Bragg L, Claringbull GF, Taylor WH. *Crystal structures of minerals. The crystalline state*. Vol 4. London: G Bell and Sons Ltd, 1965:409.
- Bunn CW. *Chemical crystallography. An introduction to optical and x-ray methods*. 2nd ed. London: Clarendon Press, 1961:509.
- Pauling L. *The nature of the chemical bond and the structure of molecules and crystals*. 3rd ed. Ithaca, New York: Cornell University Press, 1961.
- Furlong DN, Parfitt GD. Electrokinetics of titanium dioxide. *Journal of Colloid Interface Science* 1978;65:548-54.
- Knittel D. Titanium and titanium alloys. In: *Kirk-Othmer encyclopedia of chemical technology*. Vol 23. New York: Wiley-Interscience, 1982:98-130.
- Schiek RC. Pigment (inorganic). In: *Kirk-Othmer encyclopedia of chemical technology*. Vol 17. New York: Wiley-Interscience, 1982:788-838.
- Whitehead J. Titanium compounds (inorganic). In: *Kirk-Othmer encyclopedia of chemical technology*. Vol 23. New York: Wiley-Interscience, 1982:130-76.
- Solomon DH, Hawthorne DG. *Chemistry of pigments and fillers*. New York: Wiley-Interscience, 1983:51-84.