Aqueous Biphasic Hydroformylation Catalysed by Protein-Rhodium Complexes

C. Bertucci,[a] C. Botteghi,[b] D. Giunta,[c] M. Marchetti,[c,*] S. Paganelli[b]

[a] Dipartimento di Scienze Farmaceutiche, Università di Bologna, via Belmeloro 6, 40126 Bologna, Italy
[b] Dipartimento di Chimica – Università Ca' Foscari di Venezia, Calle Larga Santa Marta 2137, 30123 Venezia, Italy.
[c] Sezione di Sassari dell'Istituto di Chimica Biomolecolare – CNR, trav. La Crucca 3, Reg. Baldinca – Li Punti, 07040 Sassari, Italy
Fax: (+39) 079-396-1036, e-mail: mauro@ss.cnr.it

Received: March 12, 2002; Accepted: May 3, 2002

Abstract: The water-soluble complex derived from $Rh(CO)_2(acac)$ and human serum albumin (HSA) proved to be efficient in the hydroformylation of several olefin substrates. The chemoselectivity and regioselectivity were generally higher than those obtained by using the classic catalytic systems like TPPTS-Rh(I) (TPPTS = triphenylphosphine-3,3′,3″-trisulfonic acid trisodium salt). Styrene and 1-octene, for instance, were converted in almost quantitative yields into the corresponding oxo-aldehydes at 60 °C and 70 atm ($CO/H_2 = 1$) even at very low $Rh(CO)_2(acac)$/HSA catalyst concentrations. The possibility of easily recovering the Rh(I) compound makes the system environmentally friendly. The circular dichroism technique was useful for demonstrating the Rh(I) binding to the protein and to give information on the stability in solution of the catalytic system. Some other proteins have been used to replace HSA as complexing agent for Rh(I). The results were less impressive than those obtained using HSA and their complexes with Rh(I) were much less stable.

Keywords: aldehydes; biphasic catalysis; hydroformylation; proteins; rhodium

Introduction

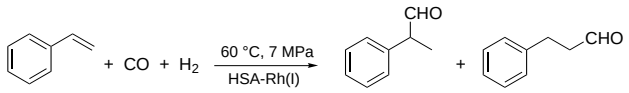
Catalysis in biphasic systems has, in the past years, undergone a remarkable development. The hydroformylation process represents one of the most striking examples of this catalytic methodology.[1–3] The use of water-soluble organometallic catalysts for chemical reactions offers considerable advantages for the chemical industry. These catalysts are so far the only successful tools for implementing the idea of heterogenisation of homogeneous catalysts by immobilising them with the aid of liquid supports.[4] They thus solve the cardinal problem of homogeneous catalysis, which lies in the expensive separation of catalyst as well as reaction product that is inherent in the system. Simple decanting the aqueous catalyst phase from the organic phase of the substrates and products separates the catalyst used in the homogeneous phase. This provides a neat and inexpensive solution to the problem of conserving resources, making the process environmentally friendly. Aqueous biphasic catalysis, however, suffers from some limitations; for instance, reaction rates are low when higher olefins are used as the substrates.[5,6]

The new processes catalysed by a variety of transition metal complexes with water-soluble ligands, therefore, constitute an outstanding application of selective homogeneous catalytic systems, as illustrated by the Ruhrchemie/Rhône-Poulenc oxo process running since 1984 (about 300,000 tons/year capacity of *n*-butanal from propene); other processes such as, for instance, the Shell SHOP process, employing homogeneous catalysis are currently being studied with the view to be converted to aqueous two-phase procedures.[7,8]. It should be pointed out that the SHOP process is currently working in a biphasic system: the ethylene oligomerisation takes place in butanediol and the oligomers are not soluble in butanediol, building the second phase. However, the introduction of a biphasic aqueous catalysis in industrial practice will represent a remarkable technical improvement.

Recently, we reported some preliminary results of a highly efficient and chemoselective olefin hydroformylation using water-soluble complexes derived from the interaction between $Rh(CO)_2(acac)$ and human serum albumin (HSA).[9] Styrene or 1-octene, for instance, were converted in almost quantitative yield into the corresponding oxo-aldehydes at 60 °C and 80 atm ($CO/H_2 = 1$) using a 600:1 substrate-to-rhodium molar ratio.[9]

In this paper we report further interesting results obtained in the hydroformylation of some representative substrates: i) in the presence of the HSA-Rh(I) catalytic system under different reaction conditions; and

  1615-4150/02/34405-556 – 562 $ 17.50-.50/0



Scheme 1. Hydroformylation of styrene.

ii) by using other protein-Rh(I) complexes like bovine serum albumin (BSA), papain, and egg albumin. Some information is also reported on the HSA binding of the metal, as obtained from circular dichroism (CD) data.

Results and Discussion

In the first phase of our research we subjected styrene, chosen as a model substrate, to the oxo reaction in the presence of the HSA-Rh(I) catalytic system; by varying some reaction parameters we hoped to gain more information on the potential of this process. Thus, styrene was hydroformylated in the presence of the complex $Rh(CO)_2(acac)$/HSA at 60 °C and 70 atm ($CO/H_2 = 1$) using increasing catalyst-to-substrates molar ratios during 24 h.

The results of three experiments are listed in Table 1. Even at an extremely low catalyst concentration, namely at a substrate-to-catalyst molar ratio = 1,000,000/1, 36% of olefin was converted to oxo-aldehydes. This value jumped to 100% at a 500,000/1 substrate-to-catalyst molar ratio, so showing the remarkable catalytic activity of this protein-Rh(I) system.

The catalytic activity of the HSA-Rh(I) solution used in Run 2 of Table 1 was seen to decrease rather rapidly after three cycles: the aldehyde yield drops from 95 to 55% (Table 2). In all experiments of Tables 1 and 2 the regioselectivity of the reaction remains practically unchanged at a branched-to-linear isomer molar ratio of about 90:10.

The results in Table 2 could be explained by taking into account that the catalytically active complex at such elevated dilutions is very readily poisoned by extremely low amounts of oxygen and/or other contaminants. This hypothesis has been supported by the results obtained when carrying out the hydroformylation of styrene under the same conditions but at a substrate-to-catalyst molar ratio 10,400:1. Indeed the activity remained, in practice, the same after six catalytic cycles, and decreased to 70% only after 10 cycles (Figure 1). The chemoselectivity was quantitative during these ten experiments, and the regioselectivity was about 95%, the branched isomer being the prevalent one.

The protein-to-metal ratio proved to be very important for the catalytic efficiency. The maximum activity was obtained only for protein-to-metal molar ratios higher than five (Table 3).

The formation of a precipitate was observed at low Rh(I)/HSA ratios: a small amount of a yellow solid product was observed for the 10/1 ratio solution. The

Table 1. Styrene hydroformylation at various HSA-Rh(I) concentrations.[a]

Run	Substrate/Catalyst	Conversion [%]	TOF/h
1	1,000,000/1	36	15,100
2	780,000/1	95	30,000
3	500,000/1	> 99	n.d.

[a] Reaction conditions: substrate = 10 mmol, reaction medium H_2O/pentane (15/5 mL), temperature = 60 °C, P = 7 MPa ($CO/H_2 = 1$), reaction time 24 h, branched-to-linear isomer ratio ≅ 90/10; starting catalytic solution: 10 mg of $Rh(CO)_2(acac)$/45 mg of HSA in 15 mL of deoxygenated water.

Table 2. Activity of recycled catalytic solutions in the hydroformylation of styrene.[a]

Run	Conversion [%]	B/L
1	95	90/10
2	88	88/12
3	55	88/12

[a] Reaction conditions: substrate = 10 mmol, reaction medium H_2O/pentane (15/5 mL), temperature = 60°C, P = 7 Mpa ($CO/H_2 = 1$), reaction time 24 h, substrate-to-catalyst molar ratio 780,000/1; starting catalytic solution: 10 mg of $Rh(CO)_2(acac)$/45 mg of HSA in 15 mL of deoxygenated water.

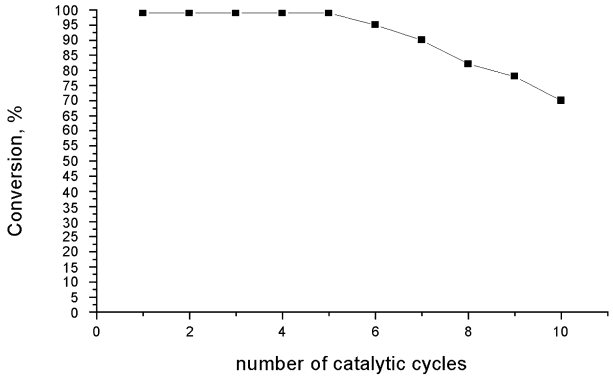


Figure 1. Dependence of the catalytic activity of HSA/Rh(I) solution on the number of cycles.

Table 3. Styrene hydroformylation at different Rh(I)/HSA ratios.[a]

Run	Rh(I)/HSA	Conversion [%]
1	1/1	0
2	3/1	75
3	5/1	> 99
4	10/1	> 99
5	30/1	> 99

[a] Reaction conditions: substrate = 10 mmol, reaction medium H_2O/pentane (15/5 mL), temperature = 60 °C, P = 7 MPa ($CO/H_2 = 1$), reaction time 24 h, substrate-to-catalyst molar ratio ca. 30,000/1.

amount of this precipitate increased in the case of a 5/1 Rh(I)/HSA ratio and it was very consistent at lower concentrations of Rh(I). This fact inhibits an efficient recycling and reuse of the catalytic solution. It is possible that 30 Rh metal atoms per HSA mol are necessary to inhibit the denaturation of the protein due to the heating.[9] In fact, the HSA-Rh(I) catalyst proved to have a high stability up to 60 °C; in experiments carried out at 100 °C a precipitate of the Rh-complex due very likely to protein denaturation was noticed.

Neutrality of the aqueous phase is necessary to reach a high catalytic activity of the HSA-Rh(I) complex in styrene hydroformylation at 60 °C and 70 atm ($CO/H_2 = 1$) (Table 4): at pH = 4 the solution becomes cloudy and formation of a white-grey precipitate is observed at the end of the reaction; moreover the water solution lost any catalytic efficiency.

The basic solution (pH 10) remains clear and no precipitate is found after the reaction; its catalytic activity is, however, practically negligible. Structural alterations of albumins have been reported to occur with temperature, this phenomenon being stronger, at least in the case of bovine serum albumin, at pH values much higher than the physiological one.[10,11]

To have a better insight on the complexation of Rh(I) to HSA, CD spectra of the $Rh(CO)_2(acac)$/HSA 20/1 complexes in buffer solution at different pH values were recorded. An induced positive CD band was observed at about 350 nm, its intensity being higher for the solution at pH 7.4 (Figure 2).

First of all, the observed CD signal demonstrates the complexation of the metal to HSA, because no intrinsic CD signal can be expected from the free $Rh(CO)_2(acac)$. Furthermore, the induced CD spectra were qualitatively different for the acid (pH 4) and basic (pH 7.4 and pH 9) solutions, the pH 4 solution showing a positive CD band at about 400 nm, in addition to the positive CD band at about 350 nm. This behaviour suggests a different stereochemistry of the HSA bound Rh(I) compound, most probably due to a different conformation of the protein at acid to neutral pH and at basic pH. Two limiting conformations are actually reported, the N and the B, the N being the prevailing one for pH values up to seven, and the B being more

Table 4. Styrene hydroformylation at different pH values.[a]

Run	pH	Conversion [%]
1	4.0	55
2	7.2	> 99
3	10.0	3

[a] Reaction conditions: substrate = 10 mmol, reaction medium H_2O/pentane (15/5 mL), temperature = 60 °C, P = 7 Mpa ($CO/H_2 = 1$), reaction time 24 h, substrate-to-catalyst molar ratio ca. 30,000/1; starting catalytic solution: 10 mg of $Rh(CO)_2(acac)$/45 mg of HSA in 15 mL of deoxygenated water.

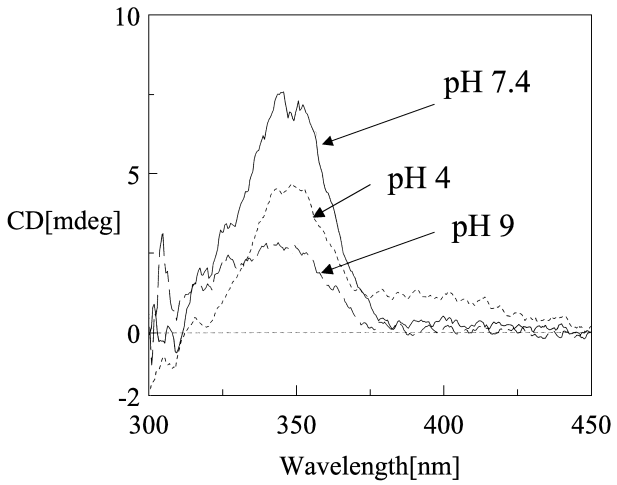


Figure 2. CD of the HSA/$Rh(CO)_2$acac 1/20 complex at pH 4, 7.4, and 9. phosphate buffer (0.1 M) solution, 1 cm cell, [HSA] 30 μM.

stable for pH ≥ 8.[12,13] At physiological pH the HSA is expected to assume both conformations.[14] This is the reported model, and it is useful to justify the incredible binding capability of albumin at physiological pH, i.e., pH 7.4, where the protein can assume both the conformations, the prevailing one depending on the structure of the ligand. As a matter of fact. the highest CD signal has been observed for the pH 7.4 solution, i.e., the pH corresponding to the highest activity of the catalyst. The smallest CD signal was observed at pH 9, and at high pH values (pH 10) the catalyst does not work at all. On the contrary, the catalytic activity was maintained at pH 4, even if a lower conversion of the substrate was observed in the hydroformylation of styrene, with respect to that at pH 7.4 (Table 4). In general, the proteins tend to have their most specific and strongest binding when working at the pH of their native environments. Working at much higher or much lower pH values will often lead to protein denaturation and a loss of specific binding. However, a relatively small pH change below or above the physiological levels can actually enhance or decrease the binding of charged solutes. For instance, HSA is a polyelectrolyte with an isoionic point of 5.2.[15] Thus, the pH should affect the net charge of the protein, in addition to the position of the conformational equilibrium between the N and B conformations.

The CD spectra were performed on the freshly prepared solutions (pH 4, pH 7.4, and pH 9) of the catalytic complexes, and after 24 hours, i.e., the reaction time for the styrene hydroformylation (Figure 3).

The 24-hour measurements were carried only for the solutions at pH 4 (Figure 3a) and pH 7.4 (Figure 3b), because at higher pH values the catalytic system is not active. The intensity of the induced CD spectra was only a little reduced after 24 h at room temperature, the reduction being more significant for the solution at

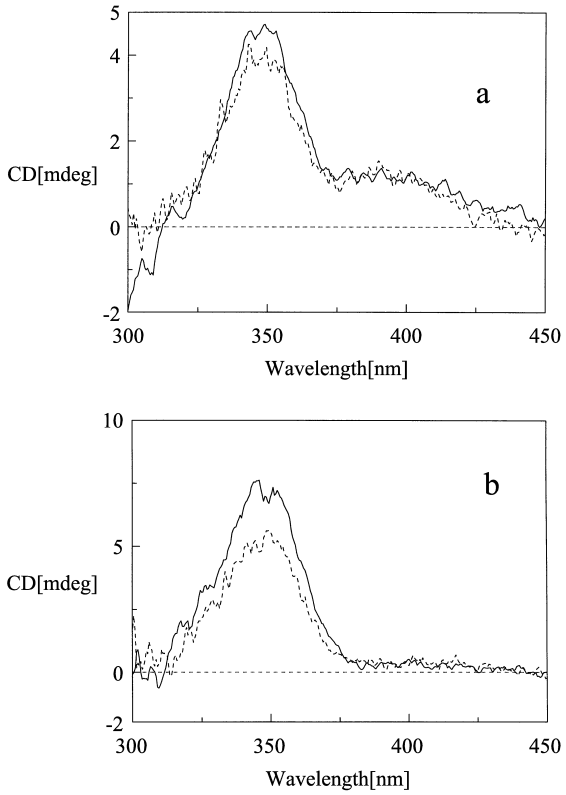


Figure 3. CD of the HSA/$Rh(CO)_2$acac 1/20 complex at pH 4 (**a**), and pH 7.4 (**b**) immediately after their preparation (——) and after 24 hours (- - - -).

pH 7.4 (Figure 3b). The relatively high stability of the catalytic species in solution does not fit with the observed degradation of the solutions in a relative short time with appearance of a precipitate. A possible explanation could be that the stereoselective binding of the Rh(I) compound to HSA occurs at a specific binding site on HSA. This high affinity binding area should be saturated because of the high excess of the metal, which then will bind also to low affinity binding sites. Thus, the metal released because of the instability of the complex can be easily replaced by the excess of the metal still bound along the protein, at lower affinity binding sites. This hypothesis, i.e., some self-regeneration of the active species of the catalyst, gives a reasonable explanation for the maintenance of the catalytic activity, in terms of substrate conversion, over five to ten cycles depending on the substrate-to-catalyst molar ratio. Thus, quite high excesses of the metal over the protein are needed to ensure a reserve of the metal and this "self-regeneration" process.

We then compared the results obtained in oxo experiments carried out under the best reaction conditions, as determined by us, of some olefinic substrates using the well known catalytic system TPPTS-Rh(I) (TPPTS = triphenylphosphine-3,3′,3″-trisulfonic acid trisodium salt) and the HSA-Rh(I) catalytic system. The results are collected in Table 5.

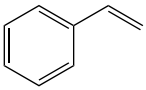
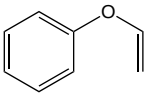
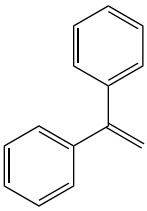
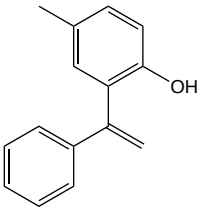
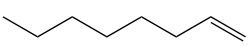
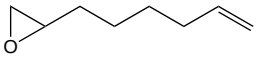
The data of Table 5 prompt the following comments: i) the chemoselectivity generally is higher using HSA-Rh(I) catalyst: for example, ethyl vinyl ether is not cleaved to the corresponding phenol; ii) the HSA-Rh(I) catalyst showed interestingly a kind of shape selectivity: 1,1-diarylethenes did not undergo hydroformylation, as found for 1,1-diphenyl-, 1,1-bis(4-fluorophenyl)-, and 1-[(2-hydroxy-5-methyl)phenyl]-1-phenylethene, which failed to react even at 100 °C and 80 atm ($CO/H_2 = 1$); iii) 1,2-epoxyoct-7-ene, an interesting precursor of valuable ω-epoxynonanal for polymer cross-linking,[16] is converted to a mixture of C_9 epoxyaldehydes (b/l = 47:53) in only 30% yield but with complete chemoselectivity; on the contrary the TPPTS-Rh(I) catalytic system produced only a complex mixture of unidentified products.

On the basis of the results presented in this paper, it can be concluded that the best conditions to achieve high yields of aldehyde in water biphasic olefin hydroformylations catalysed by the HSA-Rh(I) complex are the following: temperature: 40–60 °C, pressure = 50–70 atm ($CO/H_2 = 1{:}1$), substrate-to-catalyst molar ratio 500,000–700,000:1. The hydroformylation of styrene occurs with acceptable reaction rate even at 40 °C and 10 atm. No reaction was observed at 1 atm and 25 °C. The CD data demonstrate the binding of the metal to the protein and suggest that a high affinity binding site is involved in the stereoselective binding of the Rh(I) compound. The significant difference observed in the catalytic activity upon change of the pH of the solution should depend also on the conformation of the protein, which should change the stereochemistry of the catalytic system. As a matter of fact, when the catalytic system was prepared using denatured HSA, as obtained following a literature procedure,[17] the catalytic activity was completely lost, as shown by the fact that after 24 h at 60 °C and 70 atm ($CO/H_2 = 1$) the styrene was recovered unreacted.[17] It is worth mentioning that the ability of the protein to solubilise rhodium remained unchanged and the solution appeared clear and homogeneous.

Finally, some other readily available proteins, like papain and chicken egg albumin, were tested as ligands for Rh(I) carbonyl complexes in the aqueous biphasic olefin hydroformylation using comparable reaction conditions with respect to those employed for the HSA-Rh(I) catalytic system. Obviously, more data will be needed to optimise the experimental conditions for each protein and each substrate. Among these protein/Rh(I) complexes only styrene at 60 °C and 80 atm ($CO/H_2 = 1$) was reactive towards the hydroformylation in the presence of papain-Rh(I) and chicken egg albumin-Rh(I) complexes (Tables 6 and 7).

The obtained results suggest the following comments: i) in the case of the papain-Rh(I) system the catalytic activity decreased very rapidly and after three cycles no more activity was observed; ii) after each reaction cycle

Table 5. Comparison between the results of the aqueous biphasic hydroformylation using TPPTS-Rh(I) and HSA-Rh(I) on various substrates.[a]

Substrate	TPPTS-(Rh(I)		HSA-Rh	
	Conversion [%]	Aldehyde Yield [%]	Conversion [%]	Aldehyde Yield [%]
	95	95	> 99	> 99
	99	80[b]	70	70
	74	69[c]	–	–
	99	75[d]	–	–
	–	–	> 99	> 99
	99	–[e]	30	30

[a] Reaction conditions using the catalytic system TPPTS-Rh(I): substrate-to-Rh molar ratio 250:1, Rh/TPPTS 1:3, substrate 2.8 mmol, solvent = H_2O/toluene (2/2 mL), temperature 100 °C, $P = 5$ MPa ($CO/H_2 = 1$), reaction time 48 h. Reaction conditions using the catalytic system HSA-Rh(I): substrate = 10 mmol, reaction medium H_2O/pentane (10/5 mL), temperature = 60 °C, $P = 70$ atm ($CO/H_2 = 1$), reaction time 24 h, substrate-to-catalyst molar ratio 30,000/1; starting catalytic solution: 10 mg of $Rh(CO)_2(acac)$/45 mg of HSA in 15 mL of deoxygenated water.
[b] 19% of phenol was found among the reaction products.
[c] About 5% of hydrogenation of the substrate was observed,
[d] 24% of hydrogenation of the substrate was detected.
[e] A complex mixture of unidentified compounds was observed.

a small amount of precipitate was found and the organic solutions were coloured pale yellow, indicating that some rhodium carbonyls are extracted by the organic phase.

In the case of chicken egg albumin this effect was even more pronounced and after only one oxo experiment the catalytic solution was no longer active. At the end of the reaction the protein was completely precipitated, must probably because of its denaturation, and the organic phase was strong coloured.

Other and more specific studies on the relationship between protein structure, protein-rhodium binding type, and the catalytic activity are now in progress in our laboratories.

Experimental Section

General Methods and Chemicals

Preparative flash chromatography was performed on silica gel if not differently specified. IR spectra were measured with an FT-IR spectrometer Perkin Elmer model 1720 as KBr disks or Nujol dispersions as appropriate. Gas chromatograms were recorded with a Perkin Elmer model 8500 system, mass spectra were recorded with a GC-MS model Hewlett Packard GCD using the appropriate columns and conditions. ^1H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra of $CDCl_3$ solutions were recorded using a Varian VXR 300s spectrometer.

Styrene, 1,1-diphenylethene, 1-octene, 1,2-epoxy-7-octene, $Rh(CO)_2(acac)$, and triphenylphosphine-3,3′,3″-trisulfonic

Table 6. Aqueous biphasic hydroformylation of olefins catalysed by papain-Rh(I).[a]

Substrate	Temperature [°C]	Conversion [%]	Aldehyde Yield [%]	B/L
Styrene	60	98	100	92/8
Diphenylethene	60	–	–	–
Diphenylethene	100	8.6	75	0/100
1-Octene	60	10	100	45/55

[a] Reaction conditions: substrate = 10 mmol, reaction medium H_2O/pentane (15/5 mL), $P = 7$ MPa ($CO/H_2 = 1$), reaction time 24 h, substrate-to-Rh molar ratio *ca.* 600/1; starting catalytic solution: 10 mg of $Rh(CO)_2(acac)$/45 mg of papain in 15 mL of deoxygenated water.

Table 7. Aqueous biphasic hydroformylation of olefins catalysed by egg albumin-Rh(I).[a]

Substrate	Temperature [°C]	Conversion [%]	Aldehyde yield [%]	B/L
Styrene	40	22	100	90/10
Styrene	60	> 99	100	90/10
1-Octene	60	2	95	0/100
1,2-Epoxyundecen-10-al	60	–	–	–

[a] Reaction conditions: substrate = 10 mmol, reaction medium H_2O/pentane (15/5 mL), $P = 7$ MPa ($CO/H_2 = 1$), reaction time 24 h, substrate-to-Rh molar ratio *ca.* 600/1; starting catalytic solution: 10 mg of $Rh(CO)_2(acac)$/45 mg of egg albumin in 15 mL of deoxygenated water.

acid trisodium salt (TPPTS) were of commercial quality and used as purchased (Aldrich). Human serum albumin (HSA), papain, and egg-albumin were purchased (Sigma) and used without further purification. Phenyl vinyl ether was prepared according to a literature procedure,[18] 1,1-bis(*p*-fluorophenyl)-ethene and 1-[(2-hydroxy-5-methyl)phenyl]-1-phenylethene were synthesised as previously described.[19,20]

Circular Dichroism (CD) Measurements

CD spectra were recorded using a Jasco J-810 spectropolarimeter (Jasco, Tokyo, Japan). The instrument was interfaced to personal computers to acquire and elaborate data. All measurements were carried out at room temperature using a 1 cm path length cell. The same instrumental parameters were employed to reduce the errors: time constant 4 sec, scan speed 20 nm/min, resolution 0.2 nm, sensitivity 20 mdeg, sbw 1. The solutions of the complexes were analysed immediately after their preparation and after 24 hours to check their stability.

Preparation of HSA/Rh(I) Complex

Solutions of the HSA/$Rh(CO)_2$acac 1/20 complexes were prepared in phosphate buffer 0.1 M at pH 4, pH 7.4, and pH 9, using potassium dihydrogen phosphate, dipotassium hydrogen phosphate, phosphoric acid, and KOH to prepare and to adjust the pH of the buffer solution. The buffer was treated with N_2 to eliminate oxygen before its use for the preparation of the HSA/$Rh(CO)_2$acac solutions. In particular, $Rh(CO)_2$acac was added to a 30 μM HSA solution under stirring until a clear solution was obtained. All the used compounds were of analysis quality.

General Procedure for Aqueous Biphasic Hydroformylation in Presence of Protein-Rh(I) Complexes

The catalytically active aqueous solution was prepared by dissolving 10 mg (0.038 mmol) of $Rh(CO)_2(acac)$ in 15 mL deoxygenated water by gradual addition of 45 mg of HSA under stirring at room temperature. After 10 – 15 min a pale yellow solution was obtained and directly used as the catalytic phase for the hydroformylation of different water-insoluble olefins. The aqueous phase and a pentane solution of substrate (23 mmol in 5 mL of *n*-pentane) was introduced into a 60-mL glass tube equipped with an appropriate gas inlet-outlet cap and a magnetic stirrer bar; this glass device containing the two-phase liquid mixture was transferred to a 150-mL stainless steel autoclave under nitrogen, pressurised to 80 atm with synthesis gas ($CO/H_2 = 1$), and heated at 60 °C (substrate to rhodium molar ratio = 600:1). At the end of the reaction the organic phase was separated, pentane removed under vacuum and the aldehydes were isolated and identified as previously described.[9]

General Procedure for Aqueous Biphasic Hydroformylation in Presence of TPPTS-Rh(I) Complexes

The hydroformylations carried out in the presence of the TPPTS-Rh(I) catalysts were performed as previously described;[21] the oxo-aldehydes have been recovered and identified as described elsewhere by us.[9,21] As far as the *2-hydroxy-6-methyl-4-phenylbenzopyran-1-ol* is concerned it was recovered as a white solid by flash chromatography; m.p. 82 – 83 °C; ^{1}H NMR ($CDCl_3$): δ = 7.40 – 6.60 (m, 8H), 5.67 (t, $J = 3.05$ Hz, 2H), 4.36 – 4.28 (m, 1H), 2.34 – 2.20 (m, 2H), 2.16 (s, 3H); MS: $m/z = 240$ $[M]^+$, 222 $[M - H_2O]^+$, 195, 178, 165, 91, 77.

Hydroformylation Reaction Carried Out in the Presence of HSA-Rh(I) Complex at Various pH Values

The catalytically active aqueous solution was prepared by dissolving 10 mg (0.038 mmol) of $Rh(CO)_2(acac)$ in 15 mL deoxygenated buffer solution at the desired pH (4.0, 7.2, 10.0) by gradual addition of 45 mg of HSA under stirring at room

temperature. After 10 – 15 min a pale yellow solution was obtained and directly used as the catalytic phase for the hydroformylation of styrene. The aqueous phase and a pentane solution of substrate (23 mmol in 5 mL of *n*-pentane) were introduced into a 60-mL glass tube equipped with an appropriate gas inlet-outlet cap and a magnetic stirrer bar; this glass device containing the two-phase liquid mixture was transferred to a 150-mL stainless steel autoclave under nitrogen, pressurised to 80 atm with synthesis gas ($CO/H_2 = 1$) and heated at 60 °C (substrate to catalyst molar ratio ≈ 30,000:1). At the end of the reaction the organic phase was separated, pentane removed under vacuum, and the oxo-aldehydes were recovered and identified as previously described.[9]

Hydroformylation Reaction Carried Out at Various Concentrations of HSA-Rh(I) Complex

A catalytically active aqueous solution was prepared by dissolving 10 mg (0.038 mmol) of $Rh(CO)_2(acac)$ in 15 mL deoxygenated water by gradual addition of 45 mg of HSA under stirring at room temperature. After 10 – 15 min a pale yellow solution was obtained. A portion of this solution was diluted at the desired concentration using deoxygenated distilled water so that the final volume of water solution did not exceed 15 mL. This aqueous solution of catalyst was directly used as the catalytic phase for the hydroformylation of styrene. The aqueous phase and a pentane solution of substrate (23 mmol in 5 mL of *n*-pentane) were introduced into a 60-mL glass tube equipped with an appropriate gas inlet-outlet cap and a magnetic stirrer bar; this glass device containing the two-phase liquid mixture was transferred to a 150-mL stainless steel autoclave under nitrogen, pressurised to 80 atm with synthesis gas ($CO/H_2 = 1$) and heated at 60 °C (substrate to catalyst molar ratio ≈ 1,000,000/1, 780,000/1, 500,000/1). At the end of the reaction the organic phase was separated, pentane removed under vacuum and the oxo-aldehydes were recovered and identified as previously described.[9]

Hydroformylation Reaction Carried Out at Different Rh(I)/HSA Ratios

The catalytically active aqueous solutions were prepared by dissolving 2 mg (0.0076 mmol) of $Rh(CO)_2(acac)$ in 15 mL deoxygenated water by gradual addition of different quantities of HSA under stirring at room temperature. These aqueous solutions of catalyst were directly used as the catalytic phase for the hydroformylation of styrene. The aqueous phase and a pentane solution of substrate (23 mmol in 5 mL of *n*-pentane) were introduced into a 60-mL glass tube equipped with an appropriate gas inlet-outlet cap and a magnetic stirrer bar; this glass device containing the two-phase liquid mixture was transferred to a 150-mL stainless steel autoclave under nitrogen, pressurised to 80 atm with synthesis gas ($CO/H_2 = 1$) and heated at 60 °C (substrate to catalyst molar ratio ≈ 30,000/1). At the end of the reaction the organic phase was separated, pentane removed under vacuum and the oxo-aldehydes were recovered and identified as previously described.[9]

Acknowledgements

The authors are grateful to Mrs Barbara Sechi for the skill experimental assistance.

References

[1] B. Cornils, W. A. Herrmann (Eds.), *Aqueous-Phase Organometallic Catalysis*, VCH, Weinheim, **1999**.
[2] J. Herwig, R. Fischer, in *Rhodium Catalyzed Hydroformylation*, (Eds.: P. W. N. M. van Leuween and C. Claver), Cluver Academic Publishers, Dordrecht, **2000**, pp. 189.
[3] I. Ojima, C. Y.-Tsai,, M. Tzamarioudaki, D. Bonafoux, *Org. React*. **2000**, *56*, 1.
[4] B. Cornils, *J. Mol. Catal. A: Chemical* **1999**, *143*, 1.
[5] B. Fell, G. Papadogianakis, *J. Mol. Catal*. **1991**, *66*, 143.
[6] T. Bartik, H. Ding, B. Bartik, B. E. Hanson, *J. Mol. Catal. A: Chemical* **1995**, *98*, 117.
[7] D. Vogt, in *Aqueous-Phase Organometallic Catalysis*, (Eds.: B. Cornils, W. A. Herrmann), VCH, Weinheim, **1999**, pp. 541.
[8] Y. V. Kissing, *J. Polym. Sci., Polym. Chem. Ed*. **1989**, *27*, 147.
[9] M. Marchetti, G. Mangano, S. Paganelli, C. Botteghi, *Tetrahedron Lett.* **2000**, *41*, 3717.
[10] K. Aoki, K. Sato, S. Nagaoka, M. Kamada, K. Hiramatsu *Biochim. Biophys. Acta* **1973**, *328*, 323.
[11] R. Wetzel, M. Becker, J. Behlke, H. Billwitz, E. B. Böhm, H. Hamann, J. Krumbiegel, G. Lassmann, *Eur. J. Biochem*. **1980** *104*, 469.
[12] W. J. Leonard Jr., K. K. Vijai, J. F. Foster, *J. Biol. Chem*. **1963**, *238*, 1984.
[13] J. Wilting, M. M. Weideman; A. C. Roomer, J. H. Perrin, *Biochim. Biophys. Acta* **1979**, *579*, 469.
[14] U. Krag-Hansen, *Pharmacol. Rev.* **1981**, *33*, 17.
[15] T. Peters Jr., *All about albumin, biochemistry, genetics and medical applications*, Academic Press, New York, **1996**.
[16] C. Botteghi, M. Marchetti, S. Paganelli, S. Scognamillo, *J. Mol. Catal. A: Chemical* **2002**, *179*, 79.
[17] M. L. Elwell, J. A. Shellman, *Biochem. Biophys. Acta* **1977**, *494*, 367.
[18] C. Botteghi, G. Delogu, M. Marchetti, S. Paganelli, B. Sechi, *J. Mol. Catal. A: Chemical* **1999** *143*, 311.
[19] C. Botteghi, S. Paganelli, M. Marchetti, P. Pannocchia *J. Mol. Catal. A: Chemical* **1999**, *143*, 233.
[20] G. Sartori, S. Bigi, A. Pastorino, C. Porta, A. Arienti, R. Maggi, N. Moretti, G. Gnappi, *Tetrahedron Lett.* **1995**, *36*, 9177.
[21] S. Paganelli, M. Zanchet, M. Marchetti, G. Mangano *J. Mol. Catal. A: Chemical* **2000**, *157*, 1.