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Current Opinion in Biotechnology

Chemical speciation of trace metals in mammalian cell culture media: looking under the hood to boost cellular performance and product quality☆

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Upstream process development seeks to optimize media formulations to promote robust cell culture conditions and regulate product quality attributes such as glycosylation, aggregation, and charge variants. Transition metal ions Mn, Fe, Cu, and Zn present in cell culture media have a significant impact on cell growth, metabolism and product quality. These metals and other media components can have different chemical associations or speciation in media that are poorly characterized but may significantly impact their properties and effect on cellular performance. Computer-based equilibrium models are a good starting point for exploring metal speciation, bioavailability and conditions where precipitation may occur. However, some equilibrium constants, especially for newly introduced medium components, have not been experimentally determined. Owing to concurrent physical and biological processes, speciation may also be controlled by reaction kinetics rather than by equilibrium. These factors highlight the importance of analytically interrogating medium speciation to gain insights into the complex interconnections between media components and bioprocess performance.

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Current Opinion in Biotechnology 2021, **71**:216–224

This review comes from a themed issue on **Analytical biotechnology**

Edited by **Julian N Rosenberg**, **William E Bentley** and **Michael J Betenbaugh**

For a complete overview see the Issue and the Editorial

Available online 31st August 2021

https://doi.org/10.1016/j.copbio.2021.08.004

Introduction

Over the last four decades, various mammalian cell hosts such as murine myeloma cells (NS0), SP2/0, and more predominantly Chinese Hamster Ovary (CHO) cells, have been employed for the production of monoclonal antibodies (mAbs) and other biotherapeutic recombinant proteins used for the treatment of cancer, autoimmune disorders and various other medical conditions. CHO cells have emerged as the most preferred hosts due to their ability to produce human-like glycosylation patterns, high productivity, and robustness in scalable cell culture processes. These cells are typically grown in chemically defined serum-free media containing dozens of components critical for cell growth, protein production and modulation of product quality attributes (PQAs) such as glycosylation, aggregation, charge variants and other attributes [1].

What do we mean by chemical speciation? Within any medium containing this many components, many kinds of chemical reactions are possible. Metal ions and protons are electron pair acceptors. Protons have a coordination number of one, while media-relevant metal ions reside within coordination spheres with between four and six coordinative positions. These coordinative positions are filled with electron pair donors, termed Lewis bases or ligands. Chelating agents are molecules bearing two or more Lewis base groups capable of occupying two or more coordination positions around a central metal ion. When metal ions combine with ligands, the resulting 'species' is called a 'complex.' Chemical speciation encompasses more than just complex formation. The metal ion, and possibly coordinated ligands, can exist in two or more oxidation states. If a metal ion–ligand complex bears no charge, it can precipitate as a solid. $Fe(OH)_3$(s, ferrihydrite) serves as an illustrative example of a solid that may form in growth media. It is comprised of the metal ion Fe^{III} and the Lewis base OH^- (hydroxide ion). Coordination reactions described here are generally reversible but, depending on the metal ion and ligands involved, may be fast or slow.

☆ Given his role as Guest Editor, Michael J Betenbaugh had no involvement in the peer review of the article and has no access to information regarding its peer-review. Full responsibility for the editorial process of this article was delegated to William E. Bentley.

Speciation within extracellular media goes beyond complex formation. Two cysteine molecules can be oxidized to form cystine. Upon reduction cystine can revert back to two cysteines. Hydrogen peroxide can form an adduct with pyruvate. Rearrangement and bond cleavage within this adduct yields acetate and bicarbonate ion. In both of these examples, interconversion of reactants and products can be tracked via mass balance equations. That's why such reactions can be considered part of speciation.

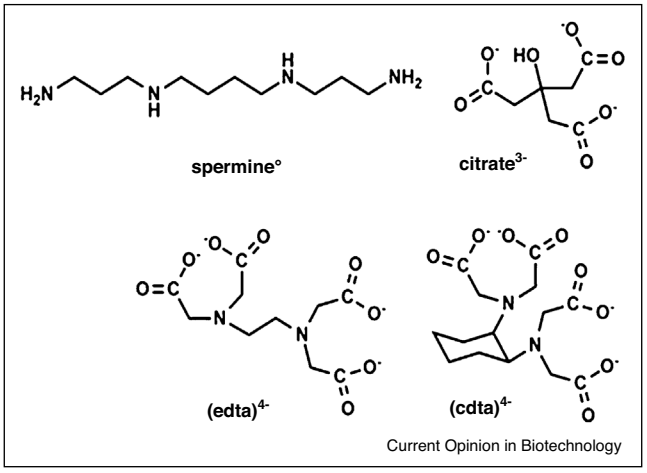
Speciation is important to how cells recognize and assimilate transition metal ion nutrients as cell culture media are often missing dedicated transporter proteins (e.g. transferrin for iron). Ultimately, chemical speciation can affect cellular uptake of nutrients, metabolism, growth and PQAs. The speciation and uptake of manganese, iron, copper, and zinc are especially important. Their bioavailability affects PQAs and extracellular metabolism via processes such as oxidative stress, lactate metabolism and apoptosis [2••]. Developing a more quantitative understanding of how the media components, especially metals, interact and impact cell growth, production, and PQAs offers predictive ability, that is, the means to interrogate a broader swath of possible media formulations than is possible experimentally, leading to superior media compositions. Elucidating chemical speciation in media formulation is a key step in designing superior media formulations. Chemical speciation, so crucial in determining bioavailability, can be under thermodynamic or under kinetic control. Hence chemical analysis to monitor total concentrations and speciation during production runs is crucial. This review outlines how knowledge of chemical speciation can aid efforts to improve nutrient assimilation and uptake, which ultimately impacts various PQAs in mammalian cell culture.

Speciation and cellular uptake

In mammals, transition metal ions are taken up by cells from blood through a multitude of mechanisms. Iron is a case in point. While erythroid cells (e.g. red blood cells) acquire Fe^{III}-transferrin via endocytosis, there are other cells that employ membrane-bound ferric reductases combined with DMT1, Zip family, or other divalent metal ion transporters [3,4]. Many animal cell culture media lack transferrin, albumin, ceruloplasmin, and other proteins that normally carry transition metal ions in blood, but cells nevertheless proliferate and biosynthesize proteins.

If a metal ion is bound too strongly to one amino acid residue within a protein transporter, it won't make it into the cytoplasm [5]. It makes sense that the most bioavailable metal ion species in the extracellular medium are 'labile', that is, capable of rapid ligand exchange. The 'free metal ion activity model' (FIAM) accurately predicts metal ion bioavailability among a wide range of organisms [6]. The FIAM model asserts that the free metal ion activity reflects the chemical reactivity of the metal and the extent to which a metal reacts with binding sites on the cell membrane surface and ultimately its bioavailability. Uptake of nutrient and toxic metal ions by neurons are now commonly investigated using 'metal ion buffers' formulated with the FIAM in mind [7,8]; by carefully selecting a chelating agent (Figure 1) with the right selectivity and logK, it is possible to fix free metal ion availability in cell cultures. In animal cell culture, FIAM is best viewed as a 'baseline' onto which (and with particular cell lines) other metal ion uptake mechanisms can be added. Indeed, mechanisms of transition metal ion uptake in culture media is an active area of investigation. Based on ionome measurements, Kropat *et al.* modified the concentrations of trace metals and introduced chelating agents such as EDTA in their growth medium resulting in faster growth, higher cell densities and better nutrient uptake by *Chlamydomonas reinhardtii* [9]. As another example, zinc concentration in CHO cell cultures has been shown to modulate cellular metal uptake as a result of altered oxidative stress defense mechanisms [10,11]. A full understanding of these mechanisms will pave the way towards improving delivery of these key cellular components.

Figure 1

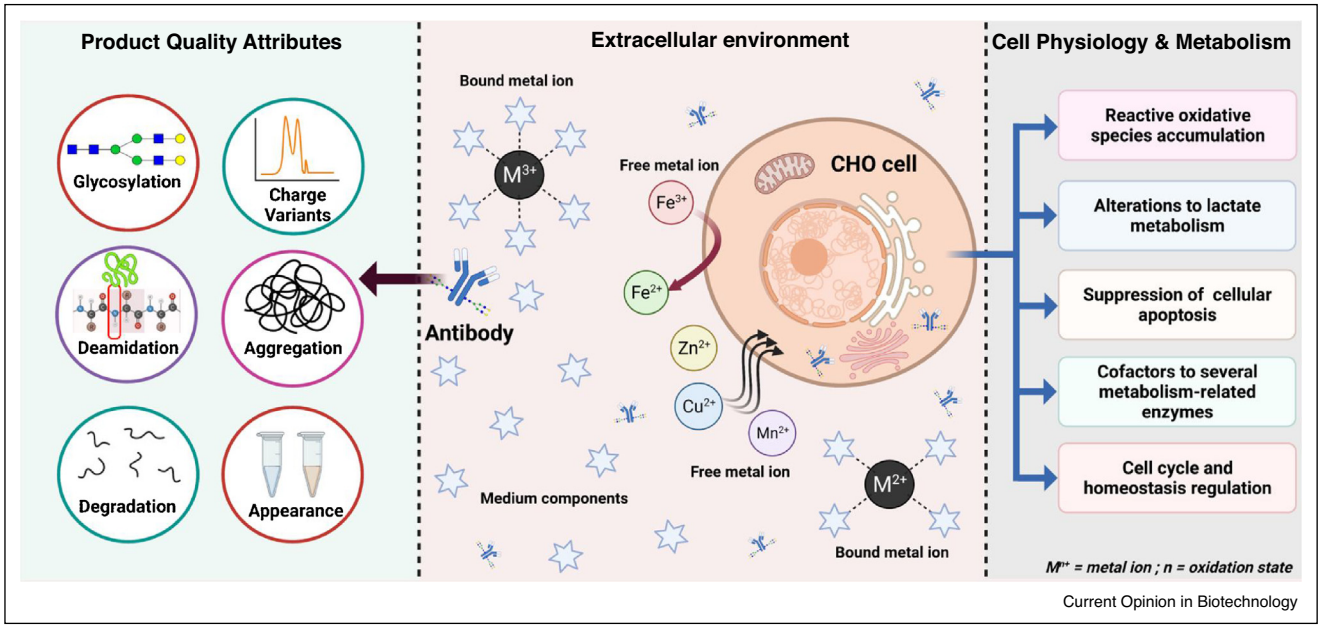


Structures of four chelating agents discussed in the text.

Impact of metal ion bioavailability on mammalian cell metabolism and product quality attributes

Once inside cells, transition metal ions are chaperoned, and ultimately delivered to target enzymes as essential cofactors. The presence or absence of these metal ion cofactors can thus have a variety of consequences on cell physiology, protein production, and important product quality attributes. It should also be noted that transition metal ions have an effect above and beyond bioavailability. They can catalyze autoxidation and lead to

Figure 2



Extracellular environment containing medium components reducing free metal availability through speciation and their impact on cell physiology, metabolism and product quality attributes. Created with BioRender.com.

Table 1

Impact of transition metal availability (Fe, Cu, Mn, Zn) on cell physiology and metabolism of mammalian cells

Trace metal	Impact on cell physiology and metabolism	References
Iron	Increased mAb productivity, enhanced environments of mitochondrial oxidative stress	[16]
	Lower ROS formation, increased peak VCD and titer in low-iron chemically defined medium	[17]
	Vital for DNA synthesis, maintaining cell homeostasis and cell cycle regulation. Its depletion induces G1/S arrest and apoptosis	[18]
	Iron-depletion leads to changes in polyamine biosynthesis, transport and catabolism	[19]
Copper	Increased copper levels led to an increased generation of free radicals and oxidative stress	[20]
	Supplementation of copper reduced lactate accumulation and downregulation of LDH gene	[21]
	Shift to net lactate consumption was observed in cultures with excess copper	[22•]
	Trafficking of PAM through the endocytic pathway is accelerated at lower copper levels and inhibited at higher copper levels	[23]
Manganese	Helps in reducing mitochondrial oxidative stress. Metal cofactor for key enzymes such as glutamine synthetase and phosphoenolpyruvate decarboxylase.	[24]
Zinc	Lower zinc levels contributed to oxidative stress, increased cell death and ROS generation	[10]
	Excess supplementation of zinc in CHO cell cultures suppressed apoptosis by reducing the caspase-3-activity	[11]

intracellular and extracellular production of reactive oxygen species (ROS). ROS production, that is, peroxides, superoxides, and hydroxyl radicals, is not only detrimental to product quality but also to cell culture performance [12]. Moreover, since trace metals also serve as cofactors for several enzymes, they are required for cellular functioning and modulation of product quality attributes such as glycosylation [13–15]. As seen in Figure 2, trace metals are involved in a wide range of metabolic processes and pathways. Table 1 summarizes the impacts of trace metal availability on CHO cell metabolism and physiology. From changes in gene expression to the cellular redox status, metal ions have a notable effect on cell growth parameters and product titers. As a result of these impacts, controlling metal ion bioavailability is vital in establishing the cell state. Table 2 outlines some of the

recent literature regarding the impact of manganese, iron, copper, and zinc on product quality attributes (PQAs). Understanding the influence of trace metals on product quality is essential as it allows for advancement in cell culture process development. Media conditions can be strategically tailored and metal concentrations optimized to achieve desired product quality attributes (e.g. correct glycosylation) while also preventing the occurrence of undesirable characteristics such as aggregation and charge variants. Resolving the role of metals and chemical speciation on these cellular processes and products are formidable tasks but also important future goals for ongoing research. Characterizing chemical speciation using quantitative equilibrium and kinetic approaches and analytical methods are the subject of the following sections.

Equilibrium concepts of speciation for media analysis

Chemical speciation is often associated with equilibria, in part because of the widespread availability of computer programs that predict equilibrium speciation by combining mass balance equations and equilibrium constants in matrix fashion [42,43••,44,45]. To achieve this goal, we employ the freeware program Visual MINTEQ [46••] which was developed for environmental and earth science applications (see Box 1). As a result, the list of chemicals from which an aqueous solution can be formulated, has to be augmented beyond the conventional set and modified to include amino acids, vitamins, and other cell growth medium-relevant components. Similarly, the equilibrium constants, reflecting quantitative species interaction at

Table 2

Impact of transition metal ions (Fe, Cu, Zn and Mn) on critical quality attributes of the biopharmaceutical protein product along with the techniques employed for their measurements

PQAs	Trace metal	Impact	Methods for measuring PQAs	Ref.
Aggregation	Iron	At lower iron concentration (0–4 ppm), proportion of high molecular weight species (HMW) was significantly lower in the presence of a chelator	HPLC-SEC (size exclusion chromatography)	[25]
		Increase in cell culture concentration of ferric ammonium citrate led to an increase in dimer concentration	HPLC-SEC	[26]
		Small dose-dependent increase in HMW species was observed with increased amounts of ferric citrate and ferric ammonium citrate in cell culture medium. No change was detected upon ammonium addition.	UPLC-SEC with UV detection	[27•]
	Copper	High levels of copper induce IgG aggregation	HPLC-SEC	[28]
		Increasing the concentration of Cu^{2+} ions increased protein aggregation of IgG fusion protein FP-A	HPLC-SEC with UV detection, HPLC-DTSEC with UV detection	[29]
		Excess CuSO4 in cell culture media resulted in a slight increase in aggregation of IgG	HPLC-SEC	[30]
		Increasing copper concentrations did not lead to aggregation	HPLC-SEC	[22•]
		Reducing the levels of copper sulfate increased the monomeric form of IgG-fusion protein B0	HPLC-SEC	[21]
	Manganese	No change was observed on changing manganese concentration	UPLC-SEC with UV detection	[27•]
Fragmentation and degradation	Iron	In the presence of histidine, iron enhanced the cleavage of human IgG	Size exclusion chromatography	[31]
	Copper	Increase in fragmentation of IgG1 with increased cupric ion concentrations especially under strong oxidizing conditions	HPLC-SEC	[32]
Charge variants	Iron	Iron increases acidic variant levels through ROS generation in the Fenton reaction	Imaged Capillary isoelectric focusing	[33]
	Copper	Increasing Cu^{2+} concentrations increased the level of proline amidation, contributing to basic charge variants	Ion exchange chromatography and Imaged capillary isoelectric focusing	[23]
		Increased Cu^{2+} concentrations decrease Trp oxidation and the overall acidic species	Imaged capillary isoelectric focusing	[34•]
		Increasing Cu^{2+} concentrations increased levels of C-terminal lysine contributing to basic charged variants	Ion exchange chromatography	[35]
	Manganese	Increased Mn^{2+} concentrations decrease Trp oxidation and the overall acidic species (Mn may be competing with Fe to prevent Fenton chemistry)	Imaged capillary isoelectric focusing and Peptide mapping using LC–MS	[34•]
	Zinc	At a particular range of concentrations (~40–130 μM) Zn^{2+} reduces levels of acidic variants and increases the main peak	Ion exchange chromatography	[36]
		Zn^{2+} (major cofactor for carboxypeptidase) decreases levels of C-terminal lysine and the overall basic species	Ion exchange chromatography	[35]
Amidation and deamidation	Iron	Deamidation of the monoclonal antibody is increased with higher iron concentration	Detected using charge heterogeneity using Imaged Capillary Isoelectric Focusing	[25]
	Copper	Higher Cu^{2+} levels in the production media increased the proline amidation levels contributing to increase in basic charge variant levels	Peptide mapping using reverse-phase LC–MS	[23]

Table 2 (*Continued*)

PQAs	Trace metal	Impact	Methods for measuring PQAs	Ref.
Glycosylation		Bioavailability of iron dictated the extent of glycosylation on the glycoprotein. The source of iron added to the culture medium was compared between Iron-EDTA and Iron-citrate	Glycosylation macroheterogeneity of IFN-γ was characterized by Western Blotting and each glycoform was quantified by densitometry	[37]
	Iron	Increased galactosylation was observed with increased iron supplementation to CHO cell cultures	Agilent 1100 HPLC system coupled with a fluorescence detector and Acquity UPLC BEH Glycan (HILIC column) (Waters) to quantify the glycan pro-file.	[16]
		Increased site occupancy of the glycoprotein	Site-occupancy of the glycoproteins was determined by reversed-phase HPLC	[38]
	Copper	Cu^{2+} ions have been reported as potent inhibitors of mannosidases I reactions	Native Gel Electrophoresis	[39]
	Manganese	Supplementation of Manganese chloride impacted the Man5 availability in CHO cells	Capillary electrophoresis with fluorescence detection (Beckman P/ ACE MDQ series and PA800)	[13]
		High mannose increases with Mn^{2+} levels in the absence of glucose or in glucose limiting conditions	MALDI-TOF using AB SCI Ex TOF/ TOF 5800 (AbSCIEx, MA, USA)	[14]
	Zinc	As the ratio between Zn^{2+} and Mn^{2+} increases on Zn^{2+} supplementation, the galactosylation level reduces due to reduced galactosyltransferase activity reduction	Released glycan analysis using MALDI TOF-MS	[40]
		Positively impacts galactosylation of mAbs. Mn^{2+} catalyzes the transfer of galactose from UDP-Gal to N-acetylglucosamine	Matrix-assisted laser desorption (MALDI-TOF) mass spectrometry	[41]

different concentrations, have to be expanded. Many equilibrium constants are available in a 2004 NIST compilation [47]. Others can be found in the literature. Visual MINTEQ can have a hundred components yielding several hundred dissolved and particulate species possible at equilibrium in typical animal cell culture media. Gas and solid phases, along with redox chemistry, can also be considered. Separate calculations can be linked in the manner of a titration, or as a 'sweep' that explores effects of changing pH or concentrations of particular components. Some species only come to the fore under high concentrations found in feed solutions, and hence may have been missed during equilibrium constant determination. Indeed, basal and feed media may contain combinations of components that prior investigators did not have a chance to thoroughly investigate. Components for which equilibrium constants have been determined may be replaced by others that are poorly documented. For example, ascorbic acid in mammalian cell growth media has largely been replaced by ascorbic acid-2-phosphate. Although the latter can be expected to complex metal ions more strongly, no corresponding logK values have been reported. Finally, some equilibrium constants are inherently difficult to measure and hence not yet determined. Redox reactions within Cu^{II}-cysteine complexes, for example, yield Cu^{I}, multiple oxidized cysteine products, and corresponding complexes, which need to be analytically resolved and quantitatively accounted for. As a result, these species may need to be characterized as described in subsequent sections.

Knowing what a metal ion is coordinated to is the first step in predicting its bioavailability. A crucial fact regarding speciation in growth media is that all amino acids possess a glycine moiety and hence are at least bidentate. The tridentate nature of aspartate, cysteine, glutamate, and histidine is readily apparent in growth media speciation. Methionine can be bound to Cu^{I} in tridentate fashion as well.

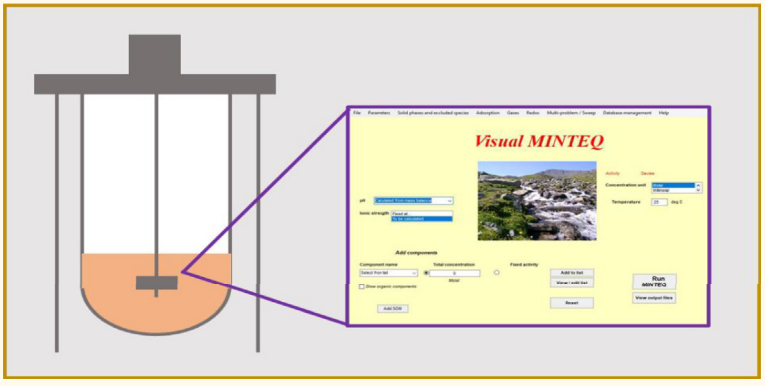
Equilibrium modelling has offered valuable insights into phenomena ranging from the behavior of transition metal ions in phloem [48] to the development of new metal-based pharmaceuticals [48,49]. The critical importance of speciation within animal cell growth media has been pointed out [50] but phenomena related to nutrient uptake and impacts on protein PQAs are largely unexplored.

Kinetic control of speciation

Not all chemical reactions in cell culture media are fast relative to such processes as nutrient uptake and metabolite release by cells, gas/water exchange, and chemical feed addition. Some chemical reactions have to 'catch up'. When slow kinetics are having an effect on speciation, we don't entirely abandon the equilibrium approach. There are two reasons for this. First, 'global' equilibrium calculations, that is, taking all possible chemical reactions into account, tell us 'Where the system is going'. Second, subsets of reactions that are sufficiently fast attain what is called 'pre-', 'quasi', or 'micro-' equilibrium relative to slow reactions. Interconversion of the ferrous iron species

Box 1 Application of Visual MINTEQ for bioreactor applications

Step 1: Add one line to the component file for the program and assign the component I.D. number.
Step 2: On running Visual MINTEQ, select the "aqueous speciation" function under "database management"
Step 3: Add reaction stoichiometries and equilibrium constants for coordinating other components. The format for reaction stoichiometry is - components only on the left-hand side of each reaction and one new "species" built from the components on the right-hand side.



Example 1: Consider the medium component spermine (sprm). In this case, we used the fully deprotonated form of spermine which has no net charge (Figure 1).

The NIST compilation lists two copper-spermine complexes.
By analogy with Cu^{II} complexes with other linear tetraamine chelating agents, a third complex $Cu(OH)(sprm)^{+}$ can be expected, but the corresponding equilibrium constant has not yet been reported:

$$Cu^{2+} + sprm^{o} = Cu(sprm)^{2+} \qquad \log K = 14.70 \qquad (1)$$

$$Cu^{2+} + sprm^{o} + H^{+} = CuH(sprm)^{3+} \qquad \log K = 20.37 \qquad (2)$$

$$Cu^{2+} + sprm^{o} + H_2O - H^{+} = Cu(OH)(sprm)^{+} \qquad \log K = ? \qquad (3)$$

Hydroxide ion (OH^{-}) is not a component but instead built from H_2O (as a default, always present) and H^{+}. While the negative sign on the left-hand side of Reaction (3) might appear troublesome, this formalism works well in the mass balance equations that ensue when the program is run.

Example 2: Citrate is an important low molecular weight chelating agent in blood and present at relatively high concentrations in typical animal cell growth media. It is traditional to represent citric acid/citrate as a tri-anion, with the alcohol group possessing a proton (Figure 1). The reasoning is that "free" citrate does not lose this proton within the pH range of natural waters and biofluids. Near neutral pH, the strong Lewis acid Fe^{III} induces deprotonation of this alcohol group, resulting in a complex with citrate that bears a -1 charge. The entry for this species within Visual MINTEQ is a bit awkward (below) but can be successfully handled during the equilibrium computation.

$$Fe^{3+} + citrate^{3-} + H_2O - H^{+} = Fe^{III}(OH)(citrate)^{-} \qquad \log K = 10.17 \qquad (4)$$

Once again, four components (left hand side) are combined to form a single new species (right hand side.) In this case, however, the "OH" in the species formula does not represent a coordinated hydroxide ion. Rather, loss of a proton by water is used as a stand-in for proton loss by the alcohol group.

Note: As electrolyte concentrations are increased, there are non-ideal interactions among ions in solution which cause equilibrium constants written in terms of concentrations to change (written as ^{c}K). For this reason, the thermodynamic database employs "infinite dilution scale" values (written as ^{a}K) which are true constants. (Using Coulomb's Law and knowledge of other physical phenomena, concentrations are converted into idealized "activities". The density of charge in three-dimensional space, termed the ionic strength, is used to calculate "activity coefficients. Visual MINTEQ and other programs perform these actions automatically).

Fe^{2+}, $Fe^{II}OH^{+}$, and $Fe^{II}(OH)_2^{\circ}$, for example, is fast relative to subsequent oxidation by O_2. (Gain or loss of a proton by coordinated hydroxide ion/water is all that is required for interconversion). Rates of reaction with O_2 decrease in the order $Fe^{II}(OH)_2^{\circ} >> Fe^{II}OH^{+} > Fe^{2+}$. Reaction via the species $Fe^{II}(OH)_2^{\circ}$ dominates, and as a consequence, autoxidation rates for 'free' ferrous ions are proportional to $[OH^{-}]^2$ [51].

Metal ion or ligand uptake by cells and addition of new feed solutions are among the many drivers of ligand exchange and metal ion exchange reactions. The chemistry of growth media constituents is such that metal ion-Lewis base bonds are usually broken and the Lewis base exits the metal ion coordination sphere before a new Lewis base takes its place. As far as pathways and rates are concerned, trends within the periodic table of the elements are most straightforward when all the coordinated ligands surrounding a central metal ion are the same and monodentate [52]. Multidentate chelating agents challenge kineticists. Copper coordinated to spermine, for example, is analogous to wearing a one-piece HazMat suit. Replacing that suit with another requires withdrawing one arm, one leg, then another, then another. Molecules have no discipline, so there is nothing to stop an arm that has exited a sleeve from reentering the sleeve. A further complication is that a 'free' arm or leg might start entering the replacement suit before the first suit has been completely exited.

We published an example of how small changes in chelating agent structure can lead to large changes in exchange rates [53••]. Our experiments started with Ni^{II} coordinated to tetradentate chelating agents NTA and EDDA, followed by addition of hexadentate chelating agents EDTA or CDTA (see Figure 1). EDTA and CDTA possess the same Lewis base groups linked to one another in the same molecular fashion. They differ in that the ethylene group bridging the two amine Lewis base groups within CDTA is rigidly held by a cyclohexane ring in a configuration 'pre-arranged' for metal ion chelation. This rigidity raises the equilibrium constant for complex formation with CDTA relative to that with EDTA. Quite interestingly, it lowers rates of Ni^{II} capture by more than an order of magnitude. Ni^{II} capture by EDTA takes minutes, while capture by CDTA takes hours. Kinetic control of the speciation of toxic levels of aluminum [54] and manganese [55] is already part of some pharmacokinetics studies. Kinetic controls on chemical speciation are considered in developing MRI contrasting agents [56] and other transition metal-based pharmaceuticals [49,50]. Approaches of this kind have great potential for animal growth media development.

Chemical analysis

Given that ligand exchange, metal ion exchange, redox, and nucleophile-electrophile reactions may be slow, chemical analytical 'ground truthing' is essential. Analytical separation before detection helps us distinguish the hundreds of species found in growth media. Capillary electrophoresis (CE) is exceptionally effective at resolving analytes. With a carefully crafted background electrolyte (BGE, the solution filling the capillary) diastereoisomers [57] and even isotopes [58] can be distinguished. To minimize changes in speciation during analysis, the BGE can be buffered to the same pH as the sample. Unlike HPLC and ion chromatography, there is no stationary phase to interact with. Timescales required for analysis can be as short as a few minutes, lessening the chance that changes in speciation have taken place, including changes brought about by what's called 'electric-field induced dissociation'. At the moment, detectors represent the biggest challenge in CE. Mass spectrometry can be expensive and difficult to maintain. Coupling CE with mass spectrometry using an ESI or APCI interface provides sensitivity and fragment information. Some caution is warranted since ionization efficiency varies considerably from analyte to analyte and chemical reactions may alter speciation during ionization. ICP-OES and IP-MS have quite successfully coupled to CE [59–61] employed in biofluid speciation studies.

Conclusions

While determinations of total concentrations of metal ions, amino acids, vitamins, and other medium components in cell culture processes are valuable, their chemical speciation is especially important owing to its impact on cell physiology, protein yield, and protein quality attributes. Equilibrium speciation calculations are a good first step in tackling the complexity of speciation within a growth medium. It is important to recognize that speciation may be under thermodynamic or kinetic control. For this reason, real-time chemical analysis of speciation is crucial. Integrating this knowledge into the bioprocess development and biomanufacturing will enable us to better track and manage the consequences of basal media composition, feed additions, and metabolic buildup on cell culture performance.

Conflict of interest statement

Nothing declared.

CRediT authorship contribution statement

Alan T Stone: Conceptualization, Writing - original draft, Writing - review & editing, Supervision. **Venkata Gayatri Dhara:** Writing - original draft, Writing - review & editing. **Harnish Mukesh Naik:** Writing - original draft, Writing - review & editing. **Lateef Aliyu:** Writing - original draft, Writing - review & editing. **Junxi Lai:** Writing - review & editing. **Jackson Jenkins:** Writing - review & editing. **Michael J Betenbaugh:** Conceptualization, Writing - review & editing, Supervision.

Acknowledgements

This work was supported by Advanced Mammalian Biomanufacturing Innovation Center (AMBIC), an Industry–University Cooperative Research Center (I/UCRC) Program under the U.S. National Science Foundation (Grant number: 1624684).

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- • of special interest
- •• of outstanding interest

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