CHAPTER 8

XAFS CHARACTERIZATION OF AYURVEDIC NANOMEDICINE

8.1 Background

The discipline of Indian Ayurvedic medicine is distinctive in that it recognizes the therapeutic potency of heavy metals or their compounds when (i) mixed with organic molecules (herbs) and (ii) processed in prescribed route employing natural products.\(^{271-275}\)

The advantages of Ayurveda over allopathic treatment include (i) cost-effectiveness (due to natural resources),\(^{276}\) (ii) longer shelf-life (due to presence of metals) and (iii) minimized adverse side effects.\(^{277}\) In India, Ayurvedic medicine assumes crucial importance in bridging the gap between heavy patient load (rural / poor) and medical accessibility.\(^{278}\) The scope of Ayurveda in India is aided by availability of abundant medicinal plants.\(^{279-281}\) Recognizing these facts, the Government of India (GOI) has taken keen interest to regularize Ayurveda as widespread alternative healthcare route. To practically realize this, the GOI has actively invested on Ayurvedic research in several institutes\(^ {282-285}\) with the objective of finding scientific evidence towards non-toxicity, standardization and effectiveness of Ayurvedic medicines.\(^ {286-287}\)

An objection raised against metal-derived drugs of Ayurveda\(^ {288-292}\) is the lack of scientific evidence for its claimed non-toxicity.\(^ {293-295}\) Some reports suggest that toxicity is neutralized by compound formation.\(^ {296}\) However, their synthesis methods do not guarantee (nor experimentally prove) total elimination of metallic phases. In the absence of scientific validation, the present criterion of toxicity (or consequent ban) is defined by the metallic content (and not the chemical or structural forms).\(^ {297}\)

The general Ayurvedic synthesis method (“Bhasmikaran”)\(^ {298-299}\) includes:
(i) Ingredients (metal + organic material): The advantages of metal (or its compounds) are manifold: longer shelf life, small attainable sizes, quick drug release, lower required dosage, size-controlled tunability of Surface Plasmon Resonance for targeted drug delivery.\(^{300}\)

(ii) Heating: The objective of heating is the formation of metal salts \([M^0 + B^0 \rightarrow M^+ (B^-); B = S, O \text{ etc.}]\). Toxic reaction of elemental metals \((M^0)\) within body \((C, H, O)\) proceeds by oxidation \([M^0 \rightarrow M^+ (C, H, O)^-]\). This is curtailed by pre-forming stable \([M^+ B^-]\) compounds before being administered into the body.\(^{301-304}\) Accomplishing 100% oxidation (zero \(M^0\) remaining) is the most crucial step for non-toxicity of the drug.

(iii) Repeated purification: Impurities / residual metals are removed by controlled heating for prolonged period of time.\(^{305}\)

(iv) Grinding – reducing the particle size such that end-product (“Bhasma”) has very fine texture (Bhasma) and no metallic shine.\(^{306-308}\)

In modern perspective, this process could be equivalent to the formation of metal-oxide (or compound) nanoparticles,\(^{309-314}\) that could act as drug-carriers in targeted drug delivery.\(^{315-316}\)

As mentioned earlier, complete oxidation and quality control of “Bhasma” particles have to be experimentally validated for Ayurvedic bhasmas to be credible and acceptable for use as drugs. Previous research includes reports on elemental analysis\(^{317}\), toxicology studies\(^{318}\), heavy metal bioaccessibility tests\(^{319}\), XRD.\(^{294,317}\) But none of them could unambiguously establish complete oxidation status or account for non-toxicity. In this work, we propose to accomplish this by XAFS-investigation of the structure of \(\alpha\)-HgS-based Rasasindura. The latter is widely prescribed (<125 mg / day) for treatment of certain diseases.\(^{320-325}\)
Elemental specificity makes XAFS\textsuperscript{46} more sensitive to small amounts (≥3%) of defects/ hidden phases that are not detected by X-Ray Diffraction (XRD). Further, XANES at Hg L3 edge contains information of the oxidation state (single or multi-valence).\textsuperscript{46} In this case, (i) in the backdrop of primary α-HgS phase, there could be minute quantities of segregated chemical phases (pure Hg, β-HgS) that are undetected by XRD; (ii) identification of core-shell (if) structure of the nanoparticles; (iii) surface segregation (if any) for the nano-crystals and (iv) identification of local defects (pores etc.) / disorder etc within the nano-particle. Each of these parameters, as explained later, is correlated with toxicity.

\textbf{8.1.1. HgS- based Rasasindura}

Due to high mobility, water-solubility and relative ease of oxidation\textsuperscript{326-327}, metallic mercury (Hg\textsuperscript{0}) is one of the most toxic elements known to human; it interacts with human body to form toxic methyl-mercury (\((CH_3)Hg\)).\textsuperscript{328} While there is worldwide concern against Hg contamination in food / water / soil\textsuperscript{329-336}, it is strange that Hg-based medicines are recommended in Ayurveda – often in concentration larger than WHO-permissible limit (> 1 ppm).\textsuperscript{337-338} From reported XRD results, the crystal structure of Rasasindura is known to be α-HgS. Preliminarily, non-toxicity of Rasasindura\textsuperscript{321} can be correlated with the advantageous properties of bulk α-HgS\textsuperscript{339}:

(i) Hg has special affinity for S\textsuperscript{340}, resulting in the formation of strong Hg-S bond. This is supported by recent experiments on Hg-contaminated soil samples\textsuperscript{57}, where it is observed that binding of Hg\textsuperscript{2+} with sulfur-containing groups (rather than oxygen-containing group) significantly arrests its reduction (Hg\textsuperscript{2+}→Hg\textsuperscript{0}).
(ii) Low solubility and bioavailability\(^{319}\) (0.001g/L) of \(\alpha\)-HgS results in low accumulation in the human body (0.2% is absorbed in the gastrointestinal tract and only 0.02% reaches the kidneys).\(^{341-342}\)

(iii) \(Toxicity[HgS] \sim 10^{-4} \times Toxicity[(CH_3)Hg]\) (Ref. 343-346)

(iv) The possibility of \(HgS \rightarrow (CH_3)Hg\) conversion by human intestinal bacteria has been ruled out.\(^{347}\)

However, these advantages may not hold good for nanoparticle form (Bhasma). Surface energetics, increased strain and vacancy defects\(^{348}\) may lead to the formation of local Hg\(^0\) sites \(i.e.\) initiate reverse reaction \([Hg^{2+} \rightarrow Hg^0]\). Further, XRD cannot unambiguously rule out the presence of < 5% Hg\(^0\) due to its limited resolution. We need to establish reliably that Rasasindura has (i) complete absence of Hg\(^0\) and (ii) robust nano-structure with minimal defects.

8.1.2. Synthesis Method

\(Rasasindura\), like any other licensed and standardised formulation, is subjected to Standard Operating Procedures (SOP), including starting / in-process / finishing quality control (QC) checks. Rasasindura was prepared by Arya Vaidya Sala (India) following three distinct steps: (i) pre-treatment of Hg and S with herbal and milk products: Hg was ground with slaked lime on a mortar for three days and filtered through a fine cloth. The filtrate was ground with garlic and rock salt until it turned black in color and then washed in water.\(^{349}\) S was heated with ghee and allowed to drop through a cloth into milk. The resultant granules were collected and washed with water\(^{350}\); (ii) mixing of Hg and S \([Hg:S = 1:1]\) along with other herbal ingredients [Aloe vera juice] and ground for five days on electric grinder, resulting in the formation of black-HgS (Kaajali)\(^{351}\); (iii) thermal treatment at 600°C of dried Kaajali, in porcelain pots, with porcelain lid and totally covered with seven layers of clay smeared cloth. The whole pot is smeared with clay for
total sealing. The pots are charged into Open Hearth Furnace (electrically operated) for 24 hours with the temperature being raised from room temperature to 600°C. After 24 hours, heating is cut off and the pots are allowed to cool down naturally for the next 24 hours. The cooled porcelain pots are opened by cutting open the clay seal. The final product, Rasasindura, in the form of fine dust of brick red colour, will be found deposited on the inside roof of the porcelain lid, which is then scraped out. It is triturated in a mortar and pestle and then passed through a fresh nylon cloth of 200-mesh.

Schematically, the entire synthesis process can be thus summarized:

\[
[(\text{Hg}) + (S)] \rightarrow \text{Kajjali(Black - HgS)} \rightarrow \text{Rasasindura(Red - HgS)}. \tag{8.1}
\]

For HgS formation, \( S + \text{Hg} \rightarrow \text{HgS}, \Delta G^\circ = -46 \text{ KJ/mol} \). The negative free energy change \( \Delta G^\circ \) shows feasibility of formation of some amount of HgS even before heat treatment of Kajjali. Heat treatment of Kajjali is essential to decrease the proportion of unreacted Sulfur, and the herbal ingredients provide the acidic medium (catalyst) required for this reaction besides aiding in solidification of Hg.

To understand the relative stability and non-toxicity of Kajjali and Rasasindura, we studied their inorganic counter-parts (viz. Black and Red HgS).

8.1.3. Inorganic Black-HgS and Red-HgS

In bulk form, Black and Red HgS generally represent the two structural forms of (inorganic) HgS:

(a) **Symmetry**: In bulk form, Black and Red HgS represent structural polymorphs viz. cubic \( \beta\text{-HgS} \) (tetrahedral) and trigonal \( \alpha\text{-HgS} \) (octahedral) respectively.
(b) **Stability**: Black $HgS$ is unstable below 600 K (Ref. 354-355), decomposes into Red $(\alpha)$-$HgS$ and is prone to oxidation. Instability of Black-$HgS$ may be due to poor packing efficiency for tetrahedral configuration.$^{xvii,356}$

On the other hand, Red $(\alpha)$-$HgS$ is reported to be stable$^{357}$ because of its significantly enhanced packing efficiency.$^{xvii}$ The smaller misfit factor for octahedral configuration in Red $(\alpha)$-$HgS$ can be accommodated with slight distortion of the octahedron and phase-stabilized.

In summary, $\alpha$ – phase $\rightarrow$ stability $\rightarrow$ non-toxicity.

(c) **Toxicity**: Red $(\alpha)$-$HgS$, the stable structural form, has not been explicitly reported as toxic in literature$^{358-360}$ $Toxicity[Red(\alpha)-HgS] - 10^{-4} \times Toxicity[(CH_3)Hg]$ (Ref. 343-346). On the other hand, there are several reports on Black-$HgS$ being toxic.$^{361}$

### 8.1.4. Pre-XAFS characterization of *Rasasindura*

Although colors are strong indicators of structural forms, they could be elusive in this case since Bhasma samples are formed of nano-sized particles where color could be size-dependent. To obtain comprehensive overview of *Rasasindura* structure (in reference to laboratory-based $HgS$), we have employed complementary techniques: XRD/ X-ray Fluorescence (XRF)/ Fourier Transform (FT)-Raman & IR/ Surface Enhanced Raman Scattering (SERS). XRD and XRF experiments were performed at Indus-2 synchrotron source (India), where high-resolution information could be extracted due to very high photon flux ($\sim 10^{11}$ photons/sec). SERS, FT-Raman and FT-IR spectra were also recorded at Bhabha Atomic Research Centre (India). The conclusions from these techniques, listed in Table 8.1, unanimously establish that *Rasasindura* has the same structure as Red $(\alpha)$-$HgS$ and additionally, is better ordered.

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$xvii$ Packing Fraction (P.F.): $P.F_{\beta-HgS} = 32\%$ , $P.F_{\alpha-HgS} = 58\%$
Fig. 8.1: Pre-XAFS characterization of *Rasasindura* in comparison with Laboratory synthesized Red HgS using: (a) XRD, (b) XRF, (c) Raman and (d) SERS.

<table>
<thead>
<tr>
<th>Technique</th>
<th><em>Rasasindura</em></th>
<th>Red HgS (lab)</th>
</tr>
</thead>
<tbody>
<tr>
<td>XRD (Fig. 8.1 (a))</td>
<td>Form</td>
<td>(\alpha)-HgS; no Hg(^0) phase</td>
</tr>
<tr>
<td></td>
<td>Shape</td>
<td>Spherical</td>
</tr>
<tr>
<td></td>
<td>Particle size</td>
<td>24nm</td>
</tr>
<tr>
<td>XRF (Fig. 8.1 (b))</td>
<td>Elements</td>
<td>Hg, S and Zn (0.6%)</td>
</tr>
<tr>
<td></td>
<td>(Hg:S) ratio</td>
<td>1:1</td>
</tr>
<tr>
<td>FT-Raman (Fig. 8.1 (c))</td>
<td>Form</td>
<td>(\alpha)-HgS</td>
</tr>
<tr>
<td></td>
<td>Bonding</td>
<td>Strong (Hg-S) bond</td>
</tr>
<tr>
<td></td>
<td>Order</td>
<td>Better ordered</td>
</tr>
<tr>
<td>SERS (Fig. 8.1 (d))</td>
<td>Surface organic groups</td>
<td>Absent</td>
</tr>
<tr>
<td>FT-IR</td>
<td>Organic groups</td>
<td>Absent</td>
</tr>
</tbody>
</table>
8.2 XAFS Experimental details

Transmission mode XAFS was recorded on *Rasasindura* and Red (α)-HgS at Hg L3 edge (12.284 keV) at Scanning EXAFS beamline (BL-09), Indus-2 Synchrotron Radiation Source, RRCAT (India). A pair of Si (111) crystals in parallel geometry was used as double crystal monochromator (DCM). 1.5 m long horizontal pre-mirror with meridionial cylindrical curvature was used to obtain collimated beam on first crystal of DCM and reject higher harmonics from the XAFS spectrum. Incident and transmitted intensities were measured using N2/Ar filled ionization chambers.

XAFS data were processed using ATHENA software. The extracted XAFS oscillations, χ(k), were Fourier-transformed into real space χ(R) for fitting. Structural model was constructed, using FEFF6.1. The model parameters were allowed to vary while fitting (using FEFFIT) to yield the best-fit values for bond lengths (R), coordination numbers (N) and Debye–Waller factors (DWF or σ²). R-factor was considered as an estimate of the quality of fit.

8.3 Results and Discussion

XAFS data for *Rasasindura* and laboratory-made Red (α)-HgS were fitted for \( k = 2.8 \pm 8.6 \, \text{Å}^{-1}; R = 1.1 \pm 3.4 \, \text{Å} \). In order to reduce error bars and have sufficient number of points for fitting, simultaneous fitting of the data was carried out for different k-weights of the transform viz. \( W(k); w = 0.1 \). Some of the ripples observed in Fig. 8.2 are due to truncation effect from limited data range and do not represent real data. Apparently, features around 2.5–3 Å (Fig. 8.2) look less sharp for *Rasasindura*. However, it should not be mis-interpreted for higher disorder; as we note from analysis results (described below), Hg-S bond-lengths in *Rasasindura* are displaced relative to
each other such that their scattering contributions phase cancel. In fact, *Rasasindura* is found to be better ordered (than Red (α)-HgS) from our analysis.

Fig. 8.2 Comparison of Hg L3 edge XAFS data on *Rasasindura* and Red (α)-HgS with simulated XAFS data for α- and β-HgS. It is clear that the data for *Rasasindura* resembles α-HgS (not β-HgS).

### 8.3.1. Phase Segregation

Since changes in XANES are very subtle between α-HgS (Hg⁺²), Hg⁰ and *Rasasindura*, we have plotted derivatives of their XANES spectra in Fig.8.3a. XANES derivative features for *Rasasindura* viz. pre-edge (A) and edge position (B) resemble α-HgS (Hg⁺²) (Fig. 8.3a) and are markedly different from Hg⁰ (C). Comparison of XANES derivative spectra of HgS compounds (*Rasasindura*, α-HgS) and Hg⁰ clearly reveals (i) shift in edge position (B) towards higher energy (E_{shift} = 5 eV) for (*Rasasindura*, α-HgS), due to higher oxidation state (+2) of Hg (ii) presence of pre-edge feature (A) in HgS due to S (2p) - Hg (6d) orbital hybridization, which is absent in Hg⁰. The main absorption edge (B) corresponds to intra-site Hg (2p) → Hg (6d) transition, consistent with dipole selection rule (∆l = ±1). While direct inter-site Hg (2p) → S (2p) transition is prohibited by dipole rule, pre-edge feature (A) is indirect signature of S (2p) through Hg (6d)-S (2p)
hybridization. Strong pre-edge (hybridization) features confirm strong Hg-S covalent bond.

Our next objective is to determine if $Hg^0$ is completely absent or partially present. Since our XRD spectrum does not show signature of $Hg^0$ phase, one can presume that $Hg^0$ content ($x$) would be $< 5\%$. On the other hand, XANES derivate spectra show that the amplitudes of $(A,B)$ peaks and their ratio $(X)$ are significantly reduced

\[
\begin{align*}
\Delta A, \\
\Delta B, \\
\Delta X
\end{align*}
\begin{pmatrix}
-33\%, \\
-25\%, \\
-17\%
\end{pmatrix}
\]

in Rasasindura $(X = 1.2$) wrt $\alpha$-HgS $(X = 1.5)$. Since peak “A” ($\alpha$-HgS) is negatively correlated with peak “C” ($Hg^0$), mixture of these phases ($Hg^0$, $\alpha$-HgS) could reduce the net amplitude around peak “A” and subsequently, reduce $X$ (as observed for Rasasindura). This implies that the reduced features of Rasasindura spectra could be consistent with the co-existence of ($Hg^0$, $\alpha$-HgS) phases. To clarify this ambiguity, we simulated the derivative spectra for different fractions ($x_{Hg^0}$) of $Hg^0$ (Fig. 8.3b). For $x_{Hg^0} = 0 \rightarrow 30\%$, amplitudes of $(A,B)$ peaks are progressively dampened while their ratio $(X = 1.5)$ remains constant. For $x_{Hg^0} \geq 30\%$, the signature of $Hg^0$ becomes dramatically conspicuous as the spectra evolve into peaks ($A', C'$) following peaks ($A,C$); peak B is completely dampened. With increasing $Hg^0$ content, the positions of ($A', C'$) respectively move away from and towards ($A,C$) such that the split becomes wider. The spectrum of Rasasindura is not consistent with any of these mixed phase features; instead, $(X = 1.3)$ resembles XANES for Hg-S bonds in soil, water etc.

Reconciling XRD and XANES results, we unambiguously conclude that $Hg^0$ is absent in Rasasindura. Absence of $Hg^0$ is the most significant evidence towards confirming non-toxicity of Rasasindura.
Fig. 8.3 Comparison between (a) derivatives of XANES data on α-HgS, Hg⁰ and Rasasindura. Rasasindura closely resembles α-HgS. Clearly in Rasasindura, (i) edge position is shifted wrt Hg⁰ (E_{shift} = 5eV) and (ii) pre-edge features are present which are absent in Hg⁰. (b) Simulated derivative spectra for different fractions (x_{Hg⁰}) of Hg^α

8.3.2. Crystalline structure details and degree of disorder

In Fig. 8.2, we compared Rasasindura / Red (α)-HgS data with simulations for α-HgS and β-HgS crystal structures. Except for larger disorder, near neighbor features (R < 2.5 Å) of both Rasasindura and Red (α)-HgS clearly resemble α-HgS (in terms of peak position). Theoretical bond-lengths for α-HgS are shown in Table 8.2. XAFS fit results (Fig. 3) conform to α-HgS configuration for S neighbors. [Other models (a) cubic = 6S (b) β-HgS = 4S (R = 2.53 Å) yielded bad fit quality.]

Fig. 8.4a: XAFS fit results for Rasasindura and Red (α)-HgS (lab). Rasasindura is more ordered than Red (α)-HgS (lab).
From Fig. 8.4, we observe that Hg-S near neighbor configuration is better ordered (lower $\sigma^2$) in *Rasasindura* compared to laboratory-synthesized inorganic Red ($\alpha$)-HgS.

<table>
<thead>
<tr>
<th>Table 8.2 Comparison of bond lengths ($R$)</th>
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<tbody>
<tr>
<td>$Hg$-$S1$</td>
</tr>
<tr>
<td>(\alpha$-HgS {theory}</td>
</tr>
<tr>
<td>Red ((\alpha)$-HgS {XAFS}</td>
</tr>
<tr>
<td>Rasasindura {XAFS}</td>
</tr>
</tbody>
</table>

In contrast, $Hg$-$Hg$ bond features ($R > 2.5\,\text{Å}$) of $\alpha$-HgS are conspicuously absent in experimental XAFS spectra\(^{369-370}\) (although XRD-generated radial distribution function (using RAD software)\(^{371}\) clearly reproduces $Hg$-$Hg$ peak). XAFS and XRD experimental results can be reconciled by considering the possible role of $Hg$-$Hg$ disorder ($\sigma^2_{Hg-Hg}$). By simulating XAFS for different $\sigma^2_{Hg-Hg}$, we determined the critical disorder value for $Hg$-$Hg$ peak suppression to be $\sigma^2_{Hg-Hg} \geq 0.025\,\text{Å}^2$ (Fig. 8.5a). Independently, we simulated XRD patterns with different values of $\sigma^2_{Hg-Hg}$. XRD pattern for *Rasasindura* is insensitive to $\sigma^2_{Hg-Hg}$ variation up to $\sigma^2_{Hg-Hg} = 0.035\,\text{Å}^2$ (Fig. 8.5b). From XAFS and XRD simulations, we thus conclude and clarify that the apparent “absence” of $Hg$-$Hg$
correlation features in experimental XAFS spectrum is actually the reflection of large disorder in \( Hg-Hg \) bonds. Despite \( Hg \) being the heavier atom, \( \sigma_{Hg-Hg}^2 \gg \sigma_{Hg-S}^2 \) due to strong \( Hg-S \) covalent bond and relatively weak Van der Waal interaction between \( Hg-Hg \). \( HgS \) structure can be depicted as spiral \(-S-Hg-S-Hg-S\)- chains (Fig. 8.6a), in which \( Hg-S \) and \( Hg-Hg \) bonds are intra- and inter- chain respectively. Any defect in the spiral (e.g. compression / elongation) affects inter-chain \( Hg-Hg \) bond substantially while strong \( Hg-S \) bond remains largely unaffected (Fig. 8.6b). This leads to \( \sigma_{Hg-Hg}^2 > \sigma_{Hg-S}^2 \).

Fig. 8.5: Simulations for different values of \( \sigma_{Hg-Hg}^2 \) : (a) XAFS simulations (b) XRD simulations. XRD spectrum is insensitive to \( \sigma_{Hg-Hg}^2 \) variation up to \( \sigma_{Hg-Hg}^2 = 0.035\text{Å}^2 \). From XAFS simulations, \( Hg-Hg \) features are suppressed for \( \sigma_{Hg-Hg}^2 \geq 0.025\text{Å}^2 \).

Fig. 8.6: (a) Structure of \( \alpha-HgS \) comprises of parallel \(-S-Hg-S-Hg-S\)- chains along c-axis. (b) Compression of one of the chains (defect) affects \( Hg-Hg \) (green arrows) substantially.
8.3.3. Analysis of the nano-crystal units

For \( D_{\text{Rasa}} = 24 \text{nm} \), surface-volume ratio of atoms is \( \sim 2\% \) i.e \( x_{\text{Hg}}^{\text{Surface}} = 2\% \). XAFS coordination result \( N_{\text{Hg-S}} = 6(\pm 3\%) \) is the site-averaged contribution from core \( (x_{\text{Hg}}^{\text{Core}} = 98\%) \) and surface \( (x_{\text{Hg}}^{\text{Surface}} = 2\%) \) sites. Considering coordination loss \( (\Delta N) \) due to truncation at bare surface, the net (theoretical) coordination for particular bond-length \( (R=\text{Hg-S} \text{ in this case}) \):

\[
N_{\text{nano}}(\text{Hg-S}) = \left[ 1 - \frac{3}{4} \left( \frac{2R}{D} \right) + \frac{1}{16} \left( \frac{2R}{D} \right)^3 \right] N_{\text{BULK}} \approx 6 \ (\approx N_{\text{exp}})
\]  

(8.2)

i.e. \( \Delta N_{p=24nm} \approx 2\% \) which is less than XAFS resolution \( (\Delta N = \pm 3\%) \). Thus, our XAFS coordination result is apparently consistent with theoretical size-dependent coordination loss for chemically homogeneous defect-free nano-crystal. Any defect, if present, has to be accommodated within 3\% error bar of XAFS coordination result. We provide defect estimates that are consistent with this constraint.

(a) Vacancy: Vacancy-induced extra coordination loss is not observed; one can rule out the presence of \( (\geq 3\%) \) vacancy in the nano-particles.

(b) Surface segregation of Hg: Due to insignificant surface-volume ratio (2\%), surface contribution to XAFS coordination result is insignificant; hence, XAFS results do not reflect surface coordination unambiguously. [We have calculated the critical core size \( (D_{\text{core}} \leq 4 \text{nm}) \) for resolving surface coordination.] To estimate the possibility (and fraction) of preferential surface site occupancy by Hg, we considered chemical non-uniformity across the nano-crystal and allowed \( (x_{\text{Hg}}^{\text{Surface}} : x_{\text{Hg}}^{\text{Core}}) \) to vary (instead of constraining \( x_{\text{Hg}}^{\text{Surface}} = 2\% \)). By re-calculating site-averaged Hg coordination as function of \( x_{\text{Hg}}^{\text{Surface}} \), we determined that \( x_{\text{Hg}}^{\text{Surface}} < 6\% \) is consistent with XAFS result.
(c) **Organic molecules at surface**: Due to insignificant surface-volume ratio and weak backscattering factor of organic elements, XAFS was unable to unambiguously detect the presence of surface organic molecules. We obtained the answer by employing SERS. SERS results convincingly ruled out the presence of organic molecule at surface. This is remarkable since Ayurvedic synthesis method involves organic molecules. We believe that organic molecules were removed during purification steps or heating.

(d) **Porosity**: To explore the presence of nano-pores (cylindrical) in the particle, we theoretically estimated the average coordination for pore parameters \((D_p, H_p, N_p)\); 

\[ D_p = \text{pore diameter}, \quad H_p = \text{pore-height}, \quad N_p = \text{number of pores}. \]

By varying one of these parameters \(H_p = 1 - 10 \, \text{nm} \) (i.e. \(H_p^{\text{max}} = \text{radius of the nano-crystal}\)), we determined the range of \((D_p, N_p)_H\) [i.e. for each \(H_p\)] that is consistent with \(N_{\text{expt}} \approx 6(\pm 3\%)\).

Compiling all these calculation results, we obtained \(D_p^{\text{max}} = 10 \, \text{nm}, N_p^{\text{max}} = 20\), \(V_p^{\text{max}} = 7\%\). [Note that \((D_p, N_p)_H\) are negatively correlated.] \(V_p^{\text{max}} = 7\% \Rightarrow \) Pores occupy small volume fraction of the nano-crystal, which is insufficient to generate large disorder and help preserve robust character of the nanoparticle. Minimal defects for these nano-crystals could be correlated with large particle size \((D_{\text{Rasa}} \approx 24 \, \text{nm})\). Interfacial defects and broadening generally increase significantly for \(D \leq 8 \, \text{nm}\).\(^{16,373-374}\)

[Note that the above conclusions for *Rasasindura* also hold good for Red (\(\alpha\)-\(\text{HgS}\) due to similar size and coordination results.] These pores could provide the pathway for drug binding.

Summary of nano-crystal coordination analysis is the presence of robust and chemically homogeneous (\(\alpha\)-\(\text{HgS}\)) nano-crystal (Fig. 8.7). Including the error bar
(\(\Delta N = \pm 3\%\)), we could accommodate possible defects in the particle (viz. vacancy, surface segregation, nano-pore) and determine the upper limit of their content: \((< 3 – 7\%)\).

Fig. 8.7: Model of Rasasindura nano-crystal with 24 nm HgS core.

8.3.4. Implications of our structural results for toxicity and synthesis route

(i) **Toxicity:** For successful non-toxic functioning of heavy-metal based Ayurvedic medicine, the most crucial factors (wrt toxicity) are (i) complete oxidation of the metals (no remnant of \(Hg^0\)-metallic state) before entering human body; (ii) integrated structure of the medicine so that oxide form is retained inside the body (no reduction to metallic form). From our work, we observe that both the criteria are met for Rasasindura.

Our main result implying non-toxicity\(^{321}\) of Rasasindura is the absence of metallic \(Hg^0\) \(\text{i.e.} Hg\) is completely oxidized]. The structural results unanimously establish that Rasasindura is composed of robust (minimal defects) single-phase \(\alpha-HgS\) nanoparticle units \(D_{\text{rasa}} \approx 24nm\). As already mentioned above, \(\alpha-HgS\) is non-toxic which implies the same for Rasasindura. Stable \(\alpha\)-phase form and robust character of the nanoparticle would help to maintain its integrity during the entire drug delivery process.\(^{375}\)
Although the implications of size ($D_{Rasa} \approx 24nm$) are not clear at the moment, we would like to cite the correlation between nano-drug and target cell sizes, as in targeted drug delivery [Optimal particle size requirement is target specific].

(ii) Synthesis: We compared the structures of Rasasindura and Red-HgS i.e. end products of organic and inorganic synthesis methods. Interestingly, we found particle size distribution is better controlled in Rasasindura. The distribution is Gaussian and size-dispersion ($\sigma_D^{Rasa} = \pm 3.5nm; 18\%$). On the other hand, size-distribution of red ($\alpha$)-HgS is non-Gaussian ($D_{peak} \approx 20nm$) and heavily skewed towards higher particle sizes; total size distribution spreads over ($\Delta D = 50nm$). Better size control in Rasasindura could be due to herbal coating - similar to the modern day surfactant- mediated nanoparticle synthesis. Coordination configuration for both show large disorder for Hg-Hg bonds but well-defined HgS bonds. Hg-S coordination distribution is better ordered in Rasasindura (Fig. 8.4) which could be due to prolonged annealing.

Thus, our work not only helps understand non-toxicity of Rasasindura but also establishes Ayurvedic synthesis method for well controlled end-product.

8.4 Conclusion

We have employed XAFS (coupled with supporting techniques) to investigate the structure of Rasasindura. The main results are that Rasasindura has the same structure as non-toxic $\alpha$-HgS and elemental $Hg^0$ is completely absent. Our results also demonstrate that the nano-crystal ($D_{Rasa} \approx 24nm$) units of Rasasindura are robust and free of organic molecules. Absence of $Hg^0$ helps in understanding of non-toxicity and the robust character implies the nano-particle integrity during drug release. Further, we observed that Ayurvedic synthesis yielded better controlled end-product than laboratory-based red
(α)-HgS: lower size dispersion and better ordered coordination configuration. With all these advantages, *Rasasindura* can be considered for potential therapeutic agent for target cell sizes (~24 nm).