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Síntesis de nanoalambres de Plata por el método del *polyol*.

Opción de titulación
Tesis

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Título del proyecto de tesis
Síntesis de nanoalambres de Plata por el método del *polyol*.

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SÍNTESIS DE NANOALAMBRES DE PLATA POR EL MÉTODO DEL *POLYOL*.

A mi familia y a todos aquellos que hicieron esto posible.

Especialmente a mis padres y hermanas, esto es para ustedes.

A José Luis Cabrera Torres, Jesús Gerardo Nava Arango, José Jaime Méndez Flores y al Dr. Rodrigo R. Velázquez Castillo.

1 ÍNDICE

2 INTRODUCCIÓN

La nanotecnología, y en general las nanociencias, son un conjunto de ramas del conocimiento que se derivan del estudio y control de la materia, su ordenamiento y composición. Los nanomateriales han sido el foco de interés en la investigación científica en las últimas dos décadas, debido a la diferencia de propiedades que demuestran éstos en bulto en contraste a escalas más pequeñas. Debido a su versatilidad y aplicaciones este tipo de materiales son considerados como una revolución en toda rama de investigación científica.

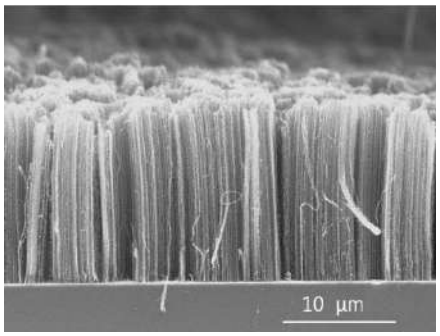


Ilustración 3 Sección de bosque de nanotubos de Carbono, tomada con SEM ~20 µm. (Dyatlova, 2012)

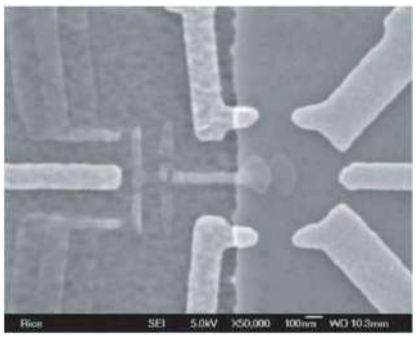


Ilustración 2 Transistor de un solo electrón. (Lu, 2003)

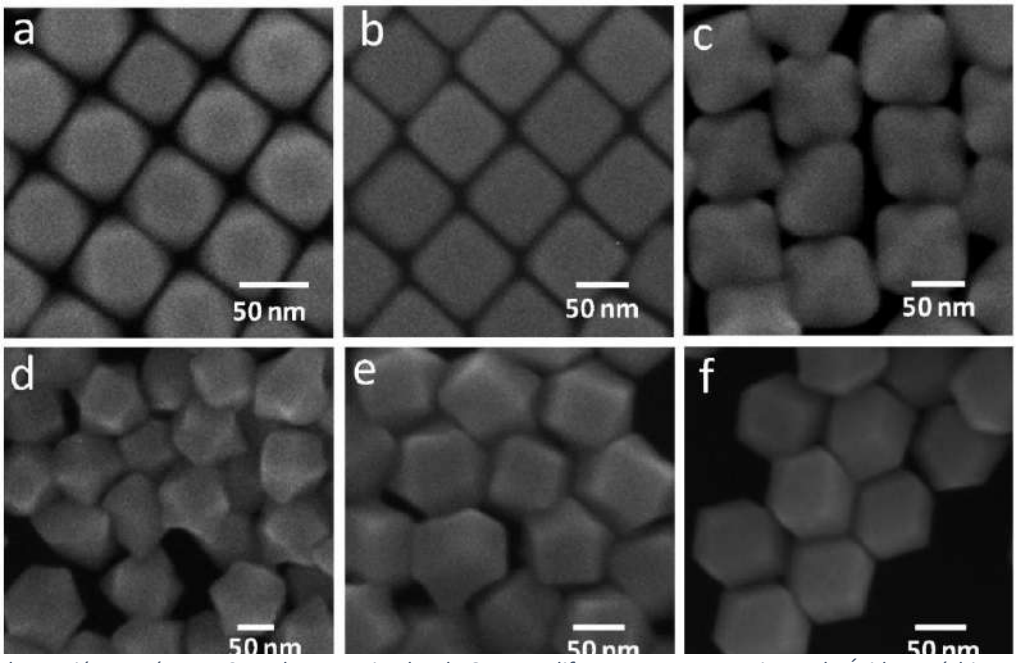


Ilustración 1 Imágenes SEM de nanocristales de Oro con diferentes concentraciones de Ácido Ascórbico para modificar su morfología. (Hsin-Lun Wu, 2010)

Para la caracterización de estas nanoestructuras, se usan métodos de microscopía electrónica de barrido (SEM), de transmisión (TEM y HRTEM), de fuerza atómica (AFM), difracción de rayos X (XRD), espectroscopía de UV-VIS y otras más.

Por lo general, a escala nanométrica, un conjunto de sustancias (partículas primarias), de diferentes formas geométricas, se ven atraídas unas a otras por interacciones físicas débiles de adhesión; el conjunto de éstas se denomina “aglomerado“, mismo que, si se ve desarrollado lo suficiente, puede formar estructuras cristalinas.

Los cristales son materiales, formados por un solo tipo de átomos o combinaciones, que tienden a ser regulares en su arreglo atómico espacial; es decir, están ordenados a un lago alcance en un patrón tridimensional repetitivo. Muchas de las propiedades de los materiales se ven afectadas por el grado de cristalinidad. Dentro de las estructuras cristalinas, a la unidad de repetición de estas se le denomina celda unidad, que son, en su mayoría, paralelepípedos o prismas. Este tipo de sistemas construyen y definen la unidad estructural fundamental del cristal; representando los desplazamientos de los átomos en unidades discretas sobre los ejes.

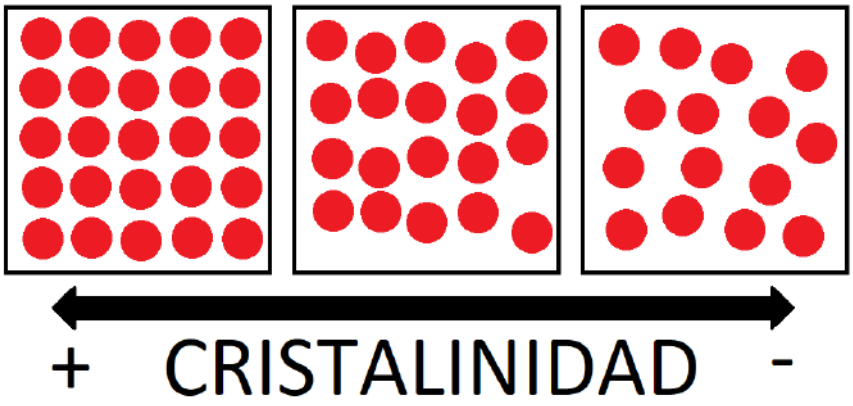


Ilustración 4 Grado de cristalización en los materiales.

El enlace atómico de los metales les confiere la cualidad de poseer muchos átomos vecinos, lo que permite un empaquetamiento denso en estructuras cristalinas. La mayoría de los metales cristalizan en: Cúbica Centrada en las Caras (FCC), Cúbica Centrada en el Cuerpo (BCC) o Hexagonal Compacta (HCP). Gracias a que las posibles combinaciones de diferentes acomodos espaciales decantan en múltiples estructuras, se clasifican en grupos de acuerdo con las configuraciones de la celda unidad.

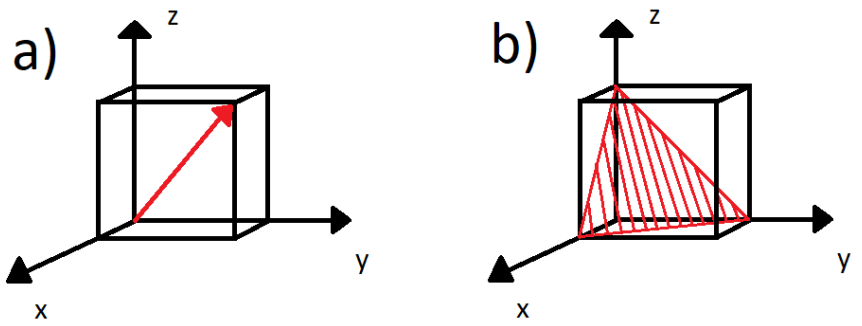


Ilustración 5 a) Dirección cristalina [1,1,1]; b) Plano cristalino (1,1,1).

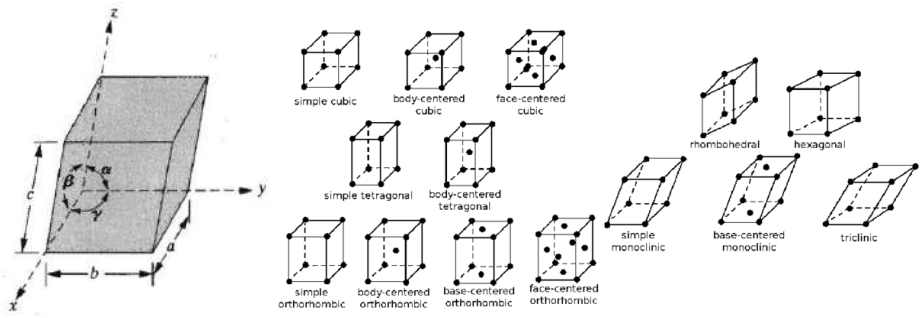


Ilustración 6 Celda unidad y las 14 redes de Bravais (Toth, 2013) (Jr., 1995).

La longitud de la celda se mide con los parámetros *a*, *b* y *c*, sobre los ejes *x*, *y* y *z*, y se definen las posiciones en el espacio tridimensional con los ángulos interaxiales *α*, *β* y *γ*. Así mismo, gracias al uso de vectores es posible trazar direcciones cristalinas dentro y fuera de la celda unidad, lo que permite una mejor orientación; estos se colocan entre corchetes *v.g.* $[001]$, $[\bar{1}10]$, etc. Para la ubicación de planos cristalográficos, se usan los índices de Miller $(\mathrm{h}, \mathrm{k}, \mathrm{l})$, donde el plano pasa por cada uno de los puntos, en referencia a los ejes (x, y, z).

El estudio de nanoestructuras metálicas, además de un interés intelectual, ha tomado auge debido a sus excelentes propiedades eléctricas, catalíticas y ópticas, mismas que son determinadas principalmente por su tamaño, morfología, composición y calidad cristalina (Cai-Xia Kan, 2008), mismos que a su vez tienen potenciales aplicaciones en campos como: sensores químico-biológicos, catálisis, fotónica, optoelectrónica y sector salud. Por sus aplicaciones, la Plata ha permitido el estudio de diversos aglomerados, entre ellos, nano-alambres, nanopartículas, nano-varillas, y otras, mismas que difieren en cualidades físico-químicas con sus equivalentes a mayor escala (en bulto). Ya que, como ha sido expresado antes, las propiedades de los materiales se ven influidos por la escala en la que se encuentran. Dentro de todas propiedades, la morfología emerge como la más influyente en el diseño de control de características en partículas metálicas.

Los metales son materiales que se caracterizan por una alta conductividad, consecuencia de un número elevado de electrones libres; dentro, los electrones de conducción son susceptibles de moverse y los de valencia se ven disminuidos, en comparación, de energía cinética. Un cristal de un metal de transición (Fe, Ag, Zn. Etc.) puede pensarse como un arreglo periódico de cargas positivas en un mar uniforme de cargas negativas, donde los electrones internos contribuyen a una elevada energía de enlace y poseen capas electrónicas *d* grandes (Kittel, 1975).

Dentro de los metales, la plata (en bulto) es el elemento que presenta la mayor conductividad térmica y eléctrica. Con el uso de estructuras uni y bidimensionales, puede verse disminuida la cantidad total de plata en sistemas que ocupan actualmente materiales en bulto o inclusive nanopartículas (*v.g.* nanoalambres, nano varillas u otras con relación de aspecto mayor) (Sun & Xia, 2002). A pesar de que existen muchas formas de sintetizar nanoalambres, la mayoría de las investigaciones se centran en mecanismos de crecimiento vía vapor-sólido o vapor-líquido-sólido, con el uso de moldes que involucran deposición química o electroquímica.

Han sido estudiadas las morfologías derivadas de la Plata y se ha encontrado que los plasmones de resonancia superficiales están fuertemente ligados a las anteriores; así: nanoalambres, nanovarillas, nanocubos, nanoesferas y demás tienen asociadas frecuencias únicas (Jie-Jun Zhu, 2011).

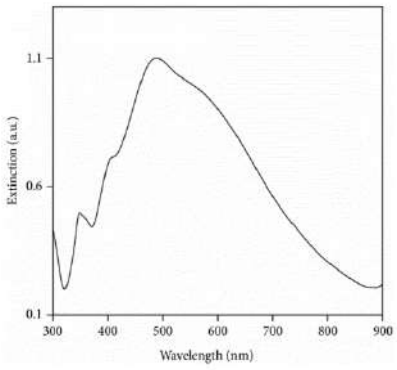


Ilustración 7 Espectro de extinción de nanocubos de Plata en etanol (Ahamad, 2014).

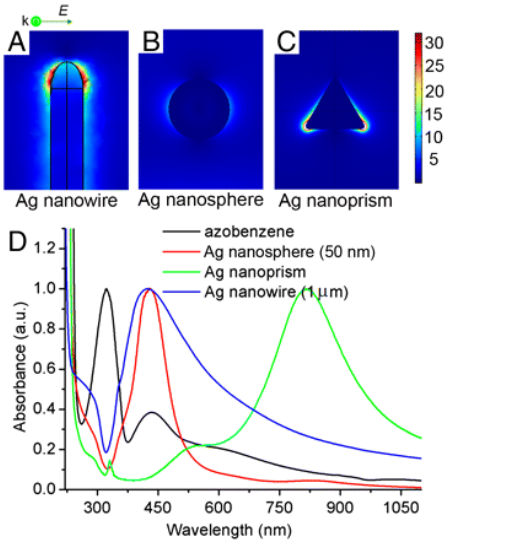


Ilustración 8 Espectro calculado de (A) Nanoalambres (B) Nanoesferas (C) Nanoprismas de Plata (Quan Yuan, 2011).

3 MÉTODO DE *POLYOL*

Este tipo de materiales han sido sintetizados usando diversos métodos, como bio-reducción, patrones y síntesis por soluciones de fase. El método del *polyol* es un procedimiento de reducción en solución, es: versátil, de bajo costo y uno de los más usados para la síntesis de nano-estructuras de Plata (y otros metales nobles), ya que éstas derivan ricas en morfología cuando se varían los parámetros de síntesis en el sistema y no es necesaria la síntesis ni presencia de semillas para iniciar el crecimiento, ya que las nanopartículas formadas al inicio de la reacción funcionan como iniciadores (Jie-Jun Zhu, 2011). Este efecto, dependiendo de las condiciones permite obtener sistemas heterogéneos, mismos que son buenos para el estudio y desarrollo intelectual, pero no tanto para aplicaciones finales de producto en industria. Además, está bien documentado que al controlar la relación molar entre el agente protector y la fuente metálica, temperatura, tiempo de reacción y orden de adición de los reactivos el producto final puede ser gobernado, en medida de lo posible, en tamaño y morfología (J. Reyes-Gasga, 2006).

El método consiste en usar como reactivos un agente reductor (Etilenglicol), precursor metálico y fuente del metal (Nitrato de Plata) y un agente protector [Polivinil-pirrolidona (PVP)], mismo que además de actuar como sitio de reducción para el catión metálico (Plata) protege al producto. Los iones de Plata Ag+ son reducidos por el Etilenglicol en presencia del mecanismo de recubrimiento que brinda la PVP; la fase de Etilenglicol-PVP no juega un papel importante en las características finales de los productos; aun así, es pertinente mencionar que el Etilenglicol funciona como agente solvente y reductor a la vez. El coloide, constituido por las partículas metálicas protegidas, forma una coordinación fuerte por medio de sorción química; dependiendo del plano de preferencia, el metal puede generar diferentes morfologías.

El Etilenglicol ($HO\text{-}CH_2\text{-}CH_2\text{-}OH$) se oxida a ($HO\text{-}CH_2\text{-}COH$), formando grupos aldehídos y agua; durante el procedimiento los iones de Plata (Ag+), presentes en la solución, se reducen, dando como resultado Ag_0. Los átomos de Plata se van uniendo, generando una estructura de tetraedro. Conforme avanza la reacción, cinco aglomerados de Plata con forma de tetraedro se unen y se forma un decaedro, que a su vez, crece en dirección a los planos preferenciales {1 1 1} longitudinalmente, formando el nano-alambre, es decir, estas nanopartículas son direccionadas para su crecimiento en nano-alambres con diámetros más o menos uniformes. Gracias al uso de PVP, se controla la velocidad de crecimiento de múltiples caras de plata gracias a la coordinación que presenta con las superficies (Sun & Xia, 2002). Finalmente, la Polivinil-pirrolidona se coloca en la superficie y extremos del nanoalambre, impidiendo su corrosión por acción del medio.

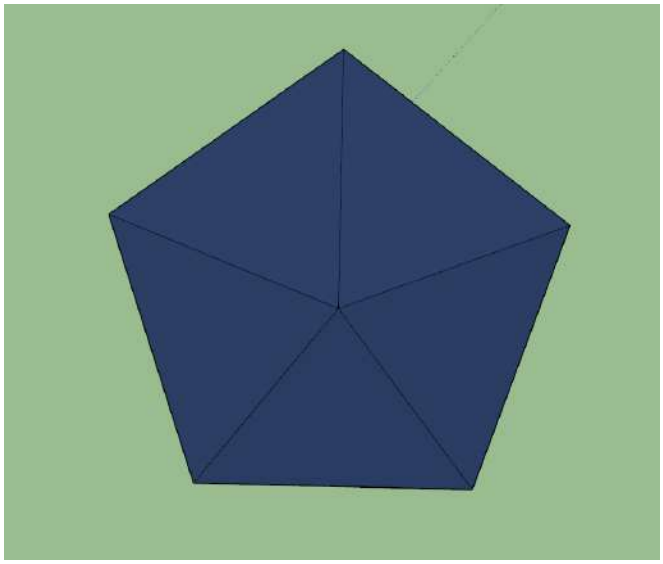


Ilustración 9 Vista esquemática frontal del decahedro formado por la Plata (Trimble Inc., 2018) .

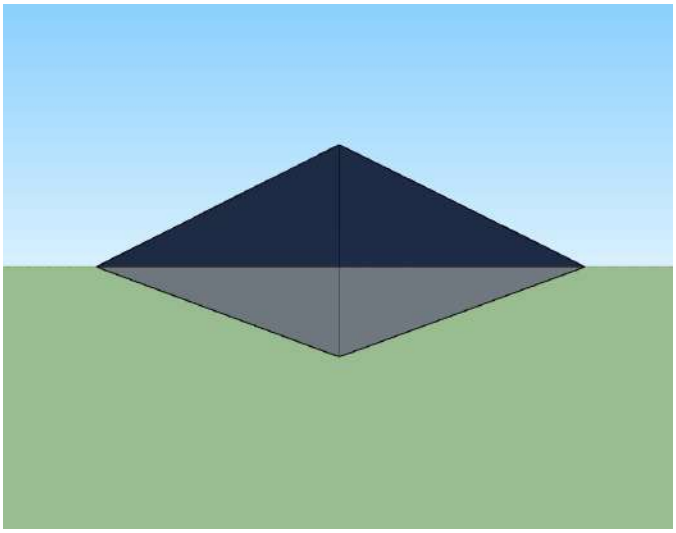


Ilustración 10 Vista esquemática lateral del decahedro formado por la Plata (Trimble Inc., 2018).

Está bastante bien documentado que las morfologías presentadas por la Plata se ven fuertemente influenciadas por la relación molar entre las unidades repetitivas de PVP y $AgNO_3$; *i.e.* al disminuir la relación molar se favorece la formación de nanoalambres con relaciones de aspecto de hasta 1000 (Tabla 1). Así mismo, el grado de polimerización de la PVP es proporcional a la cantidad final de nanoalambres (Tabla 2).

Tabla 1 Productos obtenidos a diferentes parámetros experimentales.

No	[Ag+]	R	Producto final
1	0.04 M	>15	Nanopartículas
2	0.02 M	8	Nanopartículas y algunas nanovarillas (D_{nv}) ~ 100 nm.
3	0.05 M	6	Nanoalambres y nanopartículas (muestra durante la reacción) (D_{na}) ~ 140-170 nm.
4	0.05 M	6	Nanoalambres y algunas nanopartículas (D_{nv}) ~ 100 nm.
5	0.05 M	5	Nanoalambres (D_{na}) ~ 120-150 nm.
6	0.02 M	5	Nanovarillas uniformes (D_{nv}) ~ 80 nm. L: 4-10 µm.
7	0.04 M	2.5	Nanovarillas con superficie no homogénea y nanopartículas.
8	0.04 M	1.5	Poliedros de gran tamaño, nanovarillas y cubos truncos.
9	0.04 M	1	Nanocubos y algunas nanovarillas (L_{nc}) ~ 230 nm.
10	0.04 M	0.75	Nanoalambres y algunos nanocubos (L_{nc}) ~ 140 nm.

(Cai-Xia Kan, 2008)

Aunque la acción que realiza la PVP no está muy clara, se cree que las macromoléculas de esta se tuercen y giran sobre los nanoalambres al mismo tiempo que estos crecen. Al parecer, existe una selectividad entre las superficies metálicas y los grupos funcionales del agente de coordinación. Han sido usados otros medios como Óxido de Polietileno (PEO) mismos que, aunque fueron exitosos en la síntesis de nanoalambres, los resultados fueron peores en el ámbito de uniformidad (Sun & Xia, 2002). Otras investigaciones (J. Reyes-Gasga, 2006) indican que algunas de estas nanoestructuras unidimensionales, comunes en otros metales Cúbicos Centrados en las Caras (FCC) *i.e.* Níquel, Hierro, Oro, están formadas por maclas múltiples ordenadas en pentaedros. Las terminaciones remembran decaedros y hay evidencia experimental de que existen secciones transversales por todo el eje de la nanoestructura.

Tabla 2 Productos obtenidos usando PVP con diferentes pesos moleculares (MW). (T=150°C y R=1)

No	PVP	Producto final y tamaños.
1	K17 (MW= 15000)	Nanopartículas (~60%) y nanoalambres (~40%); D: ~120 nm, L: ~3 µm.
2	K25 (MW= 38000)	Nanopartículas (~50%) y nanoalambres (~50%); D: ~100 nm, L: ~3 µm.
3	K30 (MW= 58000)	Nanopartículas (~10%) y nanoalambres (~90%); D: ~180 nm, L: ~8 µm.
4	K60 (MW= 200000)	Nanopartículas (~5%) y nanoalambres (~95%); D: ~180 nm, L: ~8 µm.
5	K90 (MW= 800000)	Nanoalambres (~100%); D: ~100 nm, L: ~10 µm.

(Jie-Jun Zhu, 2011)

4 HIPÓTESIS

Mediante el uso de dos diferentes métodos de síntesis, del control de las variables de reacción y la variación de la composición química de la mezcla de reacción, se podrá tener control sobre la morfología y las dimensiones de las estructuras nanométricas de los nanoalambres de plata, con el propósito de potencialmente obtener mejores propiedades de conducción eléctrica en el producto.

5 OBJETIVOS

OBJETIVO GENERAL

- Obtener nanoalambres de plata por el método de *polyol*.

OBJETIVOS PARTICULARES

- Lograr la síntesis de nanoalambres con alta pureza, por medio del método de *Polyol* (metodología A y B).
- Caracterizar las estructuras obtenidas por diversos métodos como microscopía electrónica de barrido y de alta resolución para su análisis estructural y cristalográfico.

6 METODOLOGÍA

Se plantea la síntesis de nano-alambres de plata, a menudo conocidos como materiales unidimensionales, dado que la relación longitud/ancho puede llegar a ser de 1000-1. Estos nano-alambres (Ag), han sido muy estudiados para aplicaciones en materiales conductores y semiconductores flexibles (Qingwen Xue, 2017), además de su uso como material de desinfección.

Estos materiales han sido sintetizados usando diversos métodos, como bio-reducción, patrones y síntesis por soluciones de fase. El método planteado en éste proyecto, Polyol, es usado en diversas nano-estructuras metálicas. El precursor metálico (típicamente sales del elemento en cuestión) se disuelve en un polyol líquido (Etilenglicol, en este caso) en presencia de un agente protector (Polivinil-pirrolidona, K90). Controlando los parámetros de reacción como la equivalencia molar entre el agente protector y el precursor metálico (AgNO3-PVP), temperatura de la reacción y el orden de adición de los reactivos, se puede tener un excelente control del tamaño y la morfología de los productos.

Todas las síntesis, diseño de experimentos, caracterizaciones y manejo de bitácoras, equipos y deshechos se realizaron de acuerdo con buenas prácticas de laboratorio y queda adjunto al final de esta tesis en el Anexo A las secciones más importantes para este tipo de trabajo, como fue requerido por el comité de Bioética de la Facultad de Ingeniería de la Universidad Autónoma de Querétaro (World Health Organization, 2009).

El proceso de síntesis se describe de forma general, ya que dentro de la experimentación fueron cambiados reactivos, así como las condiciones de reacción; incluyendo temperaturas, concentraciones, orden de reactivos y tiempo en que fueron agregadas las soluciones.

6.1.1
Método A: Nano-wires (Polyol).

Materiales:

- Matraz bola de 3 bocas.
- Agitador magnético.
- Plancha.
- Equipo de destilación.
- Plato caliente con agitación magnética.
- Tubos Falcon.
- Centrifugadora.
- Obleas de Silicio.
- 3 Vasos de precipitado 50 ml.

Reactivos:

- Nitrato de Plata ($AgNO_3$).
- Polivinil-pirrolidona (PVP).
- Etilenglicol ($C_2H_6O_2$).
- Agua desionizada.

- 1 Vaso de precipitado 250 ml.
- Espátula.
- Termómetro.
- Tapones monohoradados.
- Pipeta 5 ml.
- Perilla

Procedimiento

1. Se colocan 5ml de Etilenglicol en un vaso de precipitado de 50ml.
2. En otro vaso de precipitado de 50ml se coloca una solución de 0.36M PVP/Etilenglicol.
3. En el matraz bola de 3 bocas se hace una mezcla de Etilenglicol y la solución de PVP/Etilenglicol, calentando a 160ºC con agitación rigurosa por 60 minutos.
4. En otro vaso de precipitado de 50 ml se prepara una solución 0.12M $AgNO_3$/Etilenglicol.
5. Posteriormente se añaden 2.5ml de solución al matraz con un goteo sostenido en un periodo de tiempo igual o mayor a 6 minutos, obteniendo una tonalidad parduzca.
6. Tras un periodo de tiempo de 60 minutos la reacción se torna grisácea indicando la finalización de la misma.
7. Se deja enfriar a temperatura ambiente.

Método B: Nano-wires (*Polyol* con reflujo)

Material

- Equipo de destilación
- 2 Mangueras
- Termómetro
- Cristalizador
- Matraz bola 3 bocas
- 2 Planchas con agitación
- 3 Agitadores
- 1 Jeringa 20 mL
- 2 Jeringas 15 mL
- 2 Tapones monohoradados
- 3 Pinzas para soporte universal
- 2 Vasos de precipitado 50 mL
- 1 Vaso de precipitado de 200 mL
- 1 Perilla de succión
- 1 pipeta 5ml

Reactivos:

- 0.20 gr de $AgNO_3$
- 0.130 gr de PVP
- 20 mL de Etilenglicol (EG)

- Glicerina (calentar)

Procedimiento

1. Se conecta un tubo refrigerante y un termómetro al matraz de tres bocas.
2. En un vaso de precipitados de 50 ml se disuelve PVP en 10 ml de Etilenglicol.
3. En otro vaso de precipitados de 50 ml se disuelve $AgNO_3$ en 10 ml de Etilenglicol. Se coloca el matraz en el cristalizador a baño María en glicerina. (El tubo refrigerante ayudará a mantener la temperatura de la reacción).
4. La temperatura se estabiliza en un rango de 160ºC a 170ºC.
5. Se agrega 20ml de Etilenglicol en el matraz y se espera a estabilizar la temperatura a 160ºC con agitación moderada.
6. Esperar 30 minutos para confirmar que la temperatura es estable (160ºC).
7. Agregar ambas soluciones (PVP y AgNO*3*) en el matraz de 3 bocas con 2 jeringas, con goteo sostenido en un tiempo igual o mayor a 3 minutos, se mantiene agitación.
8. Transcurrida 1 hora se extrae con una pipeta el contenido, depositándolo en un vaso de precipitado de 250ml.

Ilustración 11 Estadios 1-3 de cambios de coloración de la reacción.

Cabe señalar que la coloración de la solución es un indicador importante en el estadio del proceso de síntesis de nanoalambres. Al inicio, la solución es transparente, ya que sólo contiene el etilenglicol que se encuentra en el proceso de

calentamiento con el objetivo de llegar a una temperatura cercana a 150 °C. Posteriormente cuando se agregan, dosificadas y al mismo tiempo, la PVP y el $AgNO_3$ la solución se torna inmediatamente amarillenta, esto indica la presencia de nanopartículas de plata en la misma (Cai-Xia Kan, 2008); debido a que ambos reactivos se encuentran a temperatura ambiente, la temperatura de la reacción baja entre 5 y 10 °C. Después del cambio de coloración amarillento, la solución cambia de color a una tonalidad más rojiza/parduzca con matices metálicos similares a las gradaciones de la Plata, que eventualmente se transforma en lechosa con precipitados negro-mate. El crecimiento y ensamble de diferentes estructuras es lo que produce el cambio.

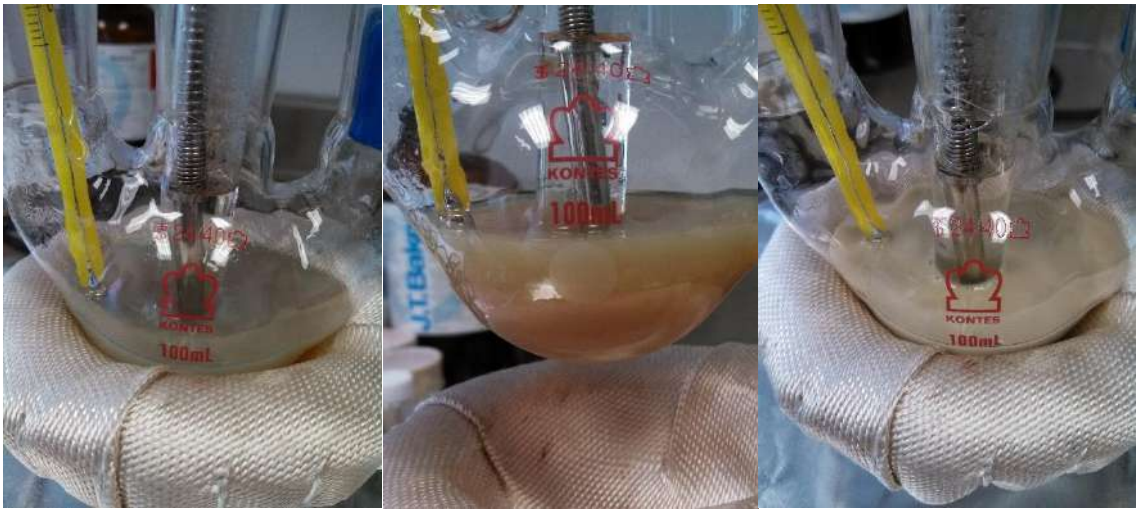


Ilustración 12 Estadios 4-6 de cambios de coloración de la reacción.

Ilustración 13 Secuencia normal de cambio de coloración.

Para la adición de los reactivos, se goteó con las dos jeringas de 20 mL al mismo tiempo directo en el matraz de tres bocas, contabilizando el tiempo con un cronómetro y controlando la temperatura de la solución general.

Ilustración 14 Adición de las soluciones de los reactivos (PVP-EG & AgNO3-EG)

Ambos procedimientos finalizan con la dispersión de los nanoalambres en etanol para eliminar el medio y obtener únicamente los nanoalambres. Posteriormente son depositados en tubos Falcon ®, mismos que se centrifugan a 4,000 revoluciones por minuto por 10 minutos hasta que el Etilenglicol no se aprecie en la muestra.

Ilustración 15 Tubos Falcon con los productos posteriores a la dispersión en Etanol.

7 RESULTADOS Y ANÁLISIS.

7.1.1 Micrografías (SEM)

La morfología de las muestras fue caracterizada con el Microscopio Electrónico de Barrido JEOL XXXXXX, con el sistema acoplado de EDS PIONEER y aditamento de bajo vacío XXXXXX perteneciente al Centro Nacional de Metrología (CENAM) en el estado de Querétaro entre agosto de 2013 y mayo de 2015.

Los resultados son presentados en esta tesis de manera cronológica, ya que así el lector podrá comprender mejor el por qué y cómo fueron tomadas las decisiones de variaciones en los parámetros de la reacción de acuerdo con los resultados obtenidos.

7.1.2 Serie 1

Al inicio, fue usado como agente reductor PVP K30 (las condiciones de reacción se especifican en la Tabla 3); este fue cambiado por PVP K90. En las primeras muestras puede observarse que el uso del reactivo con bajo peso molecular (40,000 kD), así como la inexperiencia con el método dieron como resultado un muy bajo porcentaje de nanoalambres. En su mayoría son nanopartículas no homogéneas en tamaño ni forma, con presencia de algunos nanoalambres, muy pocos y muy irregulares para ser tomados en cuenta.

Los mL en las tablas de condiciones de reacción en la categoría de AgNO3 y PVP se refieren a los mL de Etilenglicol en los cuales se disolvieron los polvos. **Estos son resultados de la reacción con metodología "A".**

Tabla 3 Condiciones de reacción, así como resumen de resultados del primer procedimiento.

EG	**$AgNO_3$**		**PVP**			**IMG**	**Temp.**	**Resultado micrografía**			**Observaciones**
mL	**mL**	**g**	**MW**	**mL**	**g**		**°C**	**NW%**	**NR%**	**NP%**	
20	10	0.5	40K	10	0.5	a-d	-	0	2	98	No existió ningún tipo de control en la reacción.

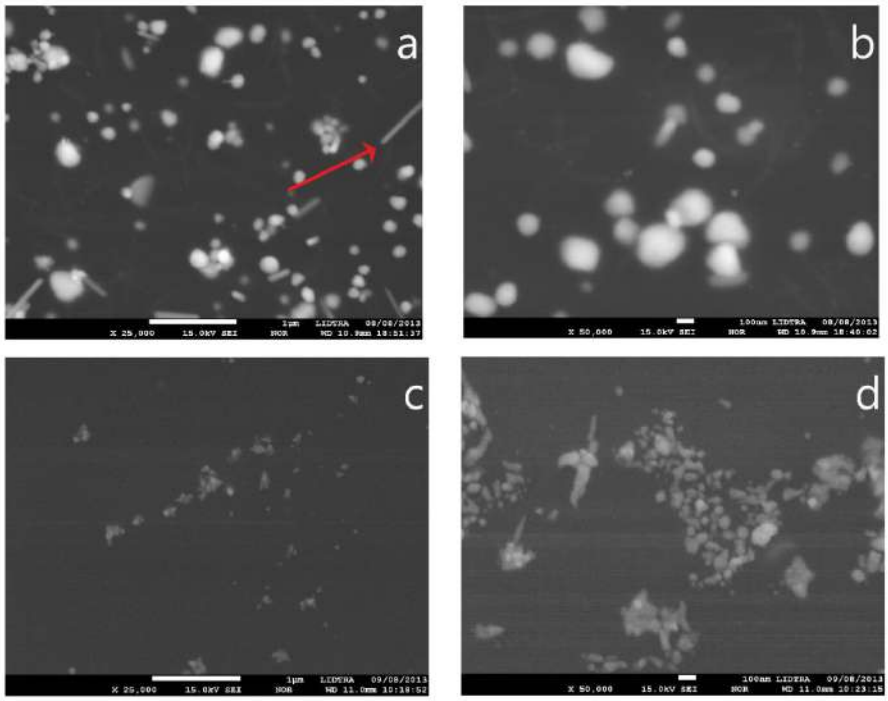


Ilustración 16 Micrografía de la primera reacción producida con PVP K30 Agosto de 2013 (SEM).

En la ilustración 16 puede observarse un nanoalambre, señalado con una flecha roja. Es evidente que el porcentaje de este tipo de estructuras en la muestra es bastante bajo, en comparación, por ejemplo a nanopartículas.

7.1.3 Serie 2

Posteriormente, y para evitar variaciones por esta razón, fue estandarizado el volumen de las reacciones a 24 mL en el matraz de tres bocas. En el segundo conjunto de reacciones se modificó la temperatura, procurando mantenerla estable en un valor para evitar variaciones por este hecho. Las temperaturas rondaron los 150 °C, usada por Jie-Jun Zhu, (2011) para sus síntesis, ya que en todos sus trabajos previos y el citado determinaron que era en la que la mayor cantidad de nanoalambres eran producidos. Los tiempos de adición fueron uniformados a alrededor de 5 minutos ± 1 minuto (esto debido a que la añadidura de los precursores fue hecha sin la ayuda de un medidor de flujo). Estos son resultados de la reacción con metodología “A”.

En un inicio se decidió usar una cantidad fija de Nitrato de Plata (0.22 g), y variar la de PVP, así como mezclar los dos pesos moleculares que se encontraban disponibles (40 y 360 KD). Cabe señalar que los procesos experimentales fueron realizados en conjuntos de tres o cuatro, lo que permitía verificar que los cambios en la reacción tenían un impacto significativo en los productos, así como evitar los posibles efectos de la oxidación en los nanoalambres.

EG	**$AgNO_3$**		**PVP**			**IMG**	**Temp.**	**Resultado micrografía**			**Observaciones**
mL	**mL**	**g**	**MW (D)**	**mL**	**g**		**°C**	**NW%**	**NR%**	**NP%**	
24	8	0.22	40K	8	1	e-h	~150	0	5 D [300 nm]	95 D [200-600 nm]	
24	8	0.22	360K	8	0.9005	i-l	~150	0	95 D [300 nm]	5 D [200 nm]	
24	8	0.222	360K	10	0.85	m-o	140	0	0	100 D [0.1-1 µm]	
24	8	0.2	360K + 40K	8	0.4/0.4	p-s	150	40 D [200 nm]	5 D [200-400 nm]	55 D [100 nm]	
24	8	0.223	360K	8	0.8884	t-w	150	70 D [250 nm]	20 D [500 nm]	10 D [500 nm]	Los nanoalambres son muy uniformes.
24	8	0.224	360K	8	0.9003	x-a*	140	0	0	100 D [150 nm]	La temperatura permaneció baja durante toda la reacción.
24	9	0.22	360K	8	0.8884	b*-e*	145	0	97 D [1 µm]	3 D [800 nm]	
24	8	0.22	360K	8	0.885	d*-g*	145	0	85 D [500-700 nm]	15 D [200-400 nm]	Aglomeración grande presente en el resultado.
24	8	0.223	360K	8	0.8884	h*-k*	150	20 D [100 nm]	0	80 D [250 nm]	Contaminaciones por material mal lavado

En la ilustración 17 puede observarse que existe un mayor número de nanoestructuras en comparación con la 15, esto probablemente es debido a la falta de control que se tuvo en la primera reacción. Hay un gran número de nanopartículas con diámetros muy superiores a los 100 nm y casi ningún

nanoalambre; la relación de cantidades entre la PVP y la fuente de Plata pudo desencadenar el que la segunda se viese cubierta con la primera sin darle oportunidad de crecer, disparando así la cantidad de nanopartículas en el producto final.

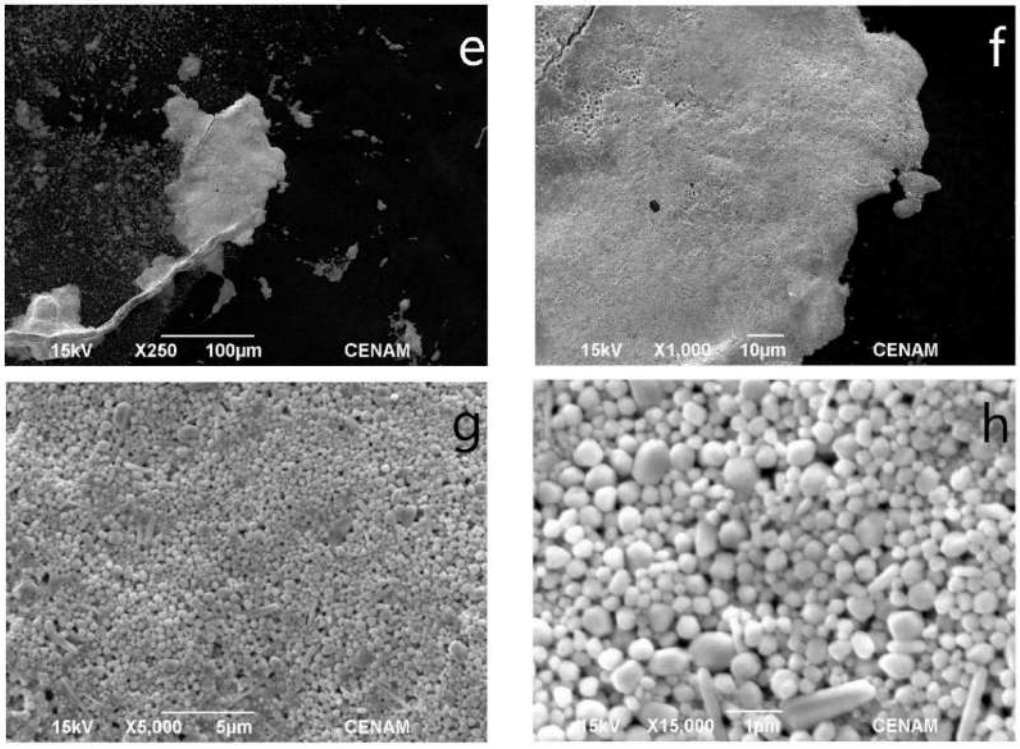


Ilustración 17 Micrografía de producto de reacción e-h.

Así mismo, en los resultados de la siguiente reacción (Ilustración 18) es notable señalar que las nanopartículas parecen nanovarillas las cuales no tuvieron tiempo de reacción o fuente de Plata suficiente para continuar con su crecimiento. Esto puede ser de utilidad para alguna otra investigación en la cual el propósito de esta sea la obtención de nanovarillas como estructura deseada. En esta, también faltó control en la temperatura, además de la gran cantidad de PVP; no obstante, el peso molecular del polímero influenció en la longitud de las nanoestructuras, ya que =se cuenta la presencia de algunos nanoalambres de alrededor de 100-200 nm (Imágenes *k* y *l*).

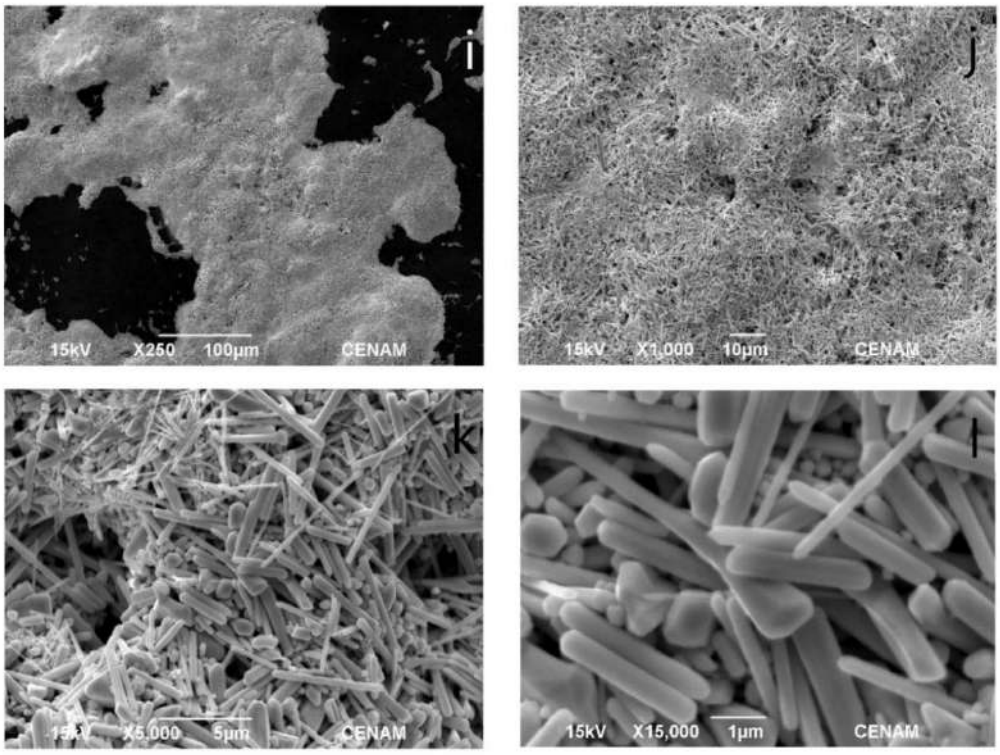


Ilustración 18 Micrografía de producto de reacción i-l.

Del resultado de la síntesis Ilustración 19 y 23, fue determinado que la temperatura de la reacción no podía ser menor o igual a 140 °C, ya que, en la primera, las temperaturas fluctuaron desde 150 hasta 140 °C, en la segunda, se mantuvieron valores cercanos a 140 durante la síntesis, con la finalidad de evidenciar que, aunque en ambos casos fue usado el polímero de alto peso molecular, el resultado son únicamente nanopartículas.

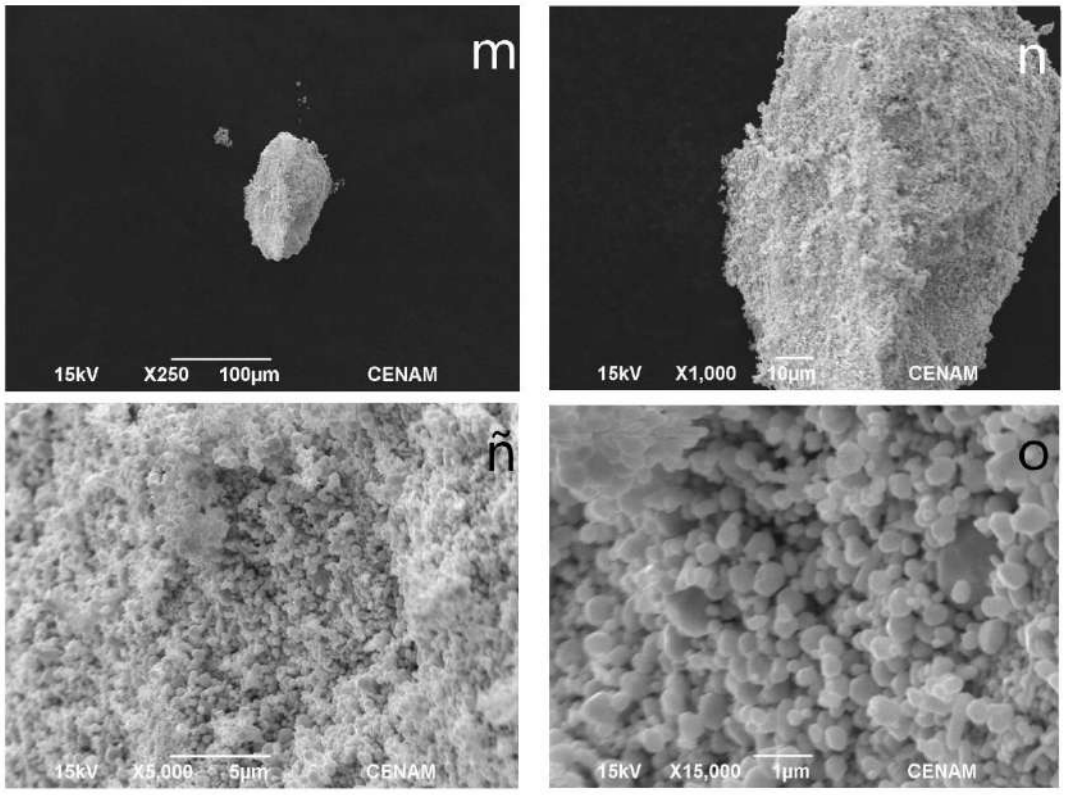


Ilustración 19 Micrografía de producto de reacción m-o.

Puede observarse en la Ilustración 20 que hay una proporción casi 1:1 de nanopartículas y nanoalambres, con poca o nula presencia de nanovarillas; esto concuerda con el hecho de que el PVP K30 se usa para sintetizar nanopartículas y el K90 para nanoalambres [de acuerdo con (Jie-Jun Zhu, 2011)]. Durante la misma existió buen control de la temperatura, lo cual confirma que el manejo de esta variable permite la obtención de productos homogéneos.

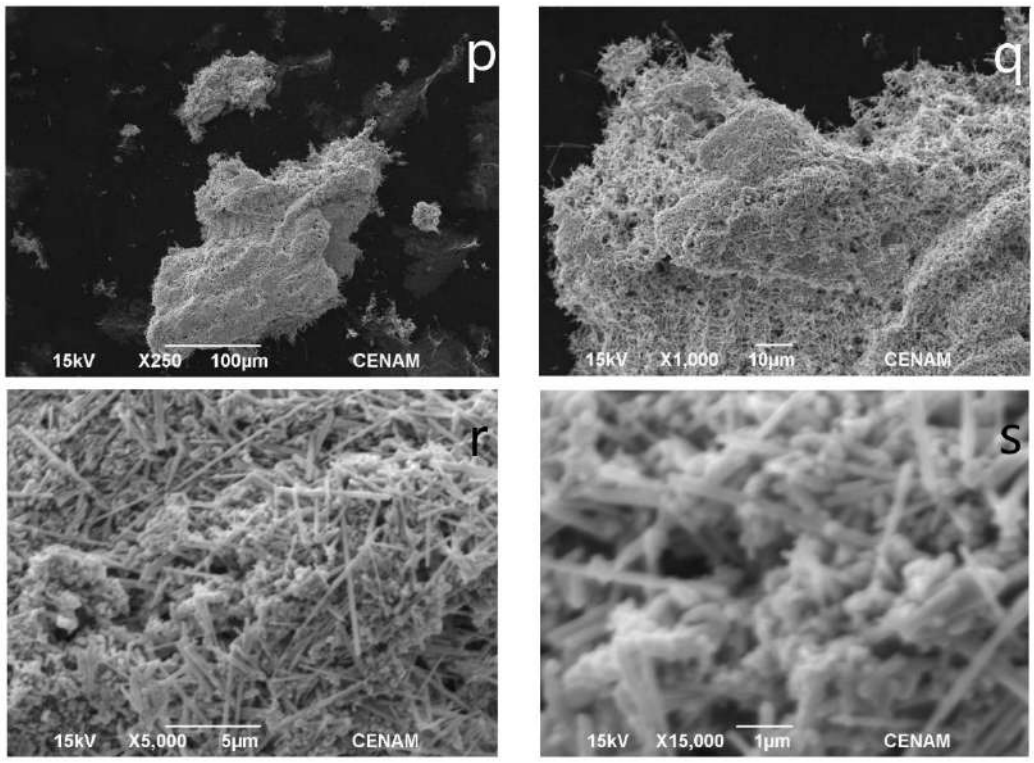


Ilustración 20 Micrografía de producto de reacción p-s.

Es posible que, de la primera serie de reacciones, la mejor de todas sea la expresada en la Ilustración 21; esta fue el patrón elegido para la siguiente secuencia de reacciones por su relación de nanoalambres (70%), nanovarillas (20%) y nanopartículas (10%).

Las condiciones concuerdan con las expresadas por (Rodrigo Rafael & Miguel, 2010), en estas también podemos observar los mismos tipos de crecimiento bidimensionales, propósito final de esta tesis (Comparativa 1). En su trabajo, se menciona que con estas mismas condiciones es posible obtener este tipo de estructuras. En la Comparativa 2 puede verse del lado derecho la frontera de grano a partir de la cual creció el nanoalambre en otra dirección cristalina.

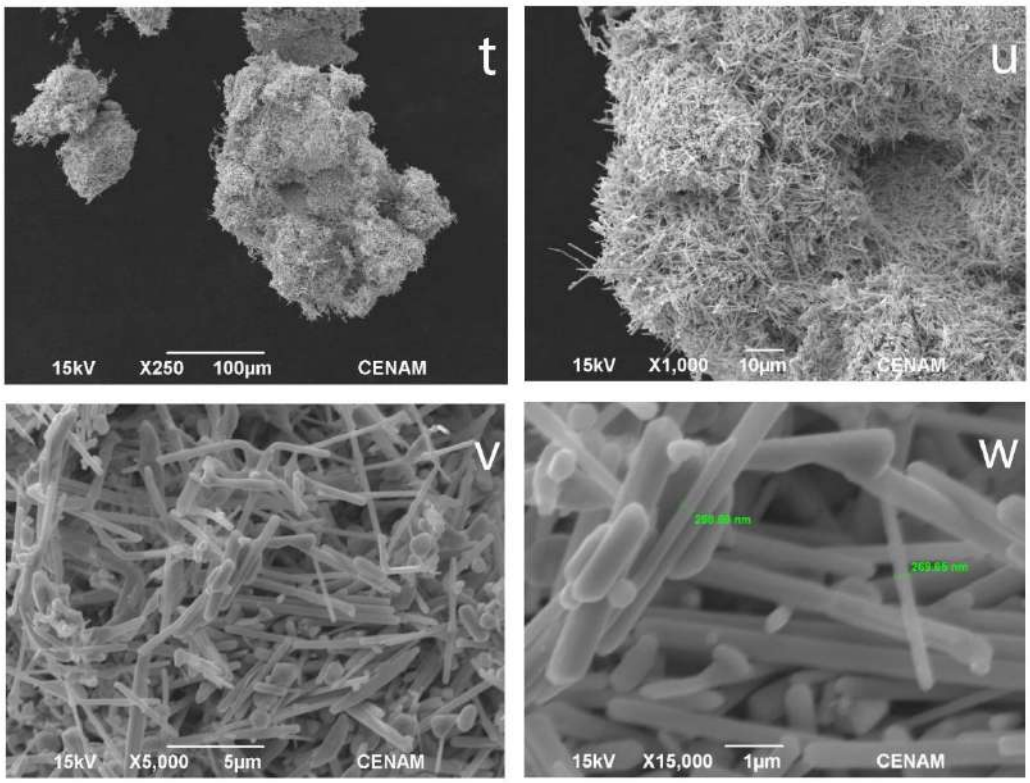
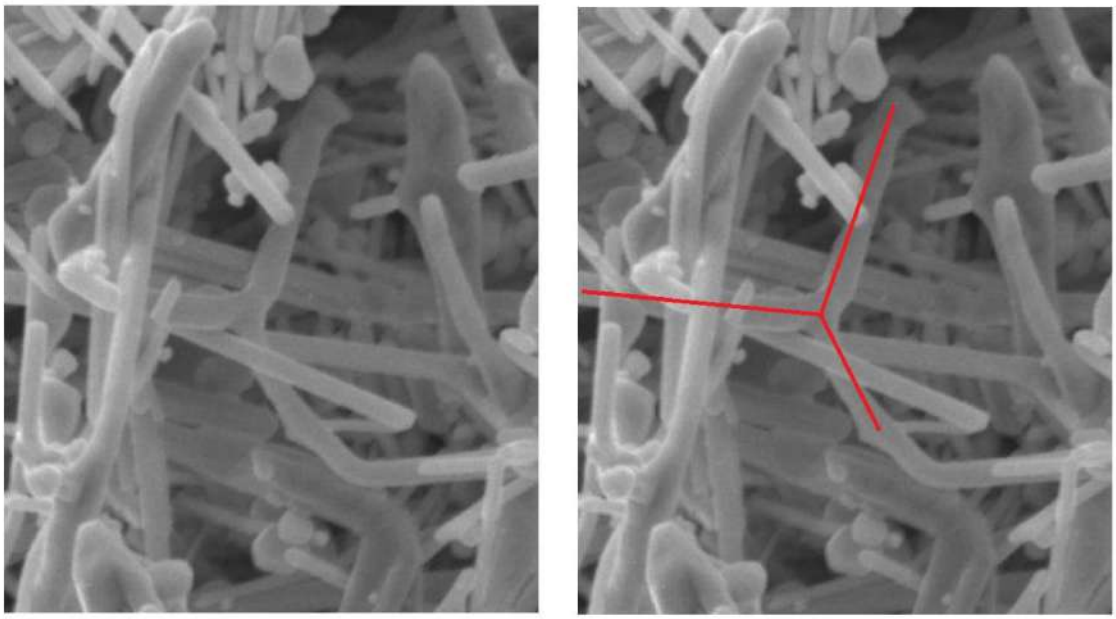
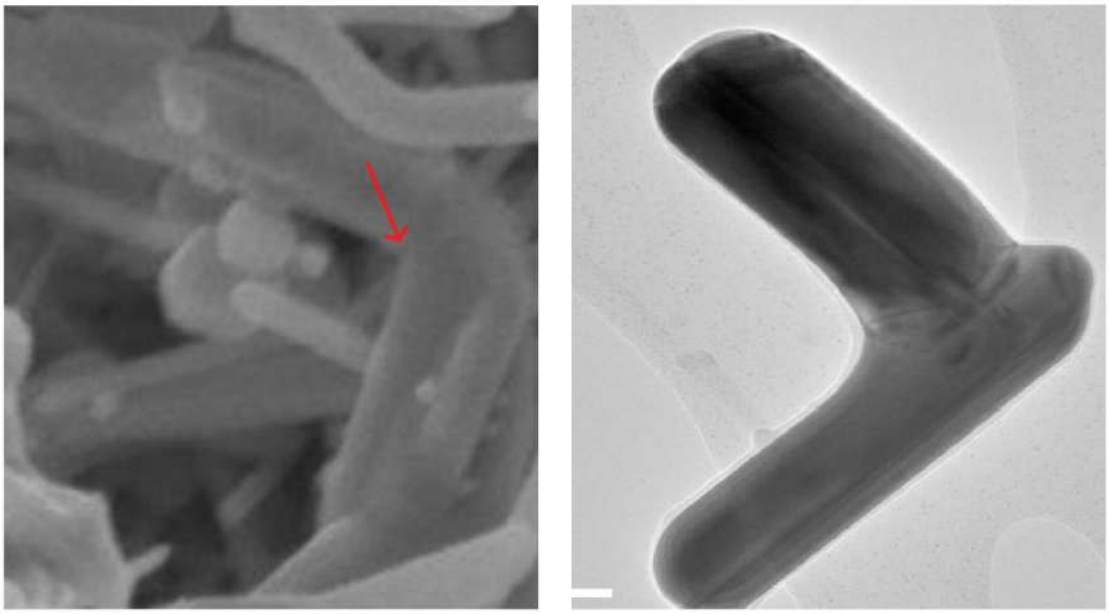


Ilustración 21 Micrografía de producto de reacción t-w.



Comparativa 1 Muestra de la presencia de nanoalambres de Plata bidimensionales.



Comparativa 2 Izquierda, SEM de muestra 20, la flecha roja señala la posible frontera de grano. Derecha, SEM de (Rodrigo Rafael & Miguel, 2010) mostrando claramente la frontera de grano.

Este tipo de crecimientos se ha propuesto que son debido a que nanopartículas se adhieren a la pared del nanoalambre, permitiendo la bifurcación del mismo y el crecimiento cristalino en esa dirección.

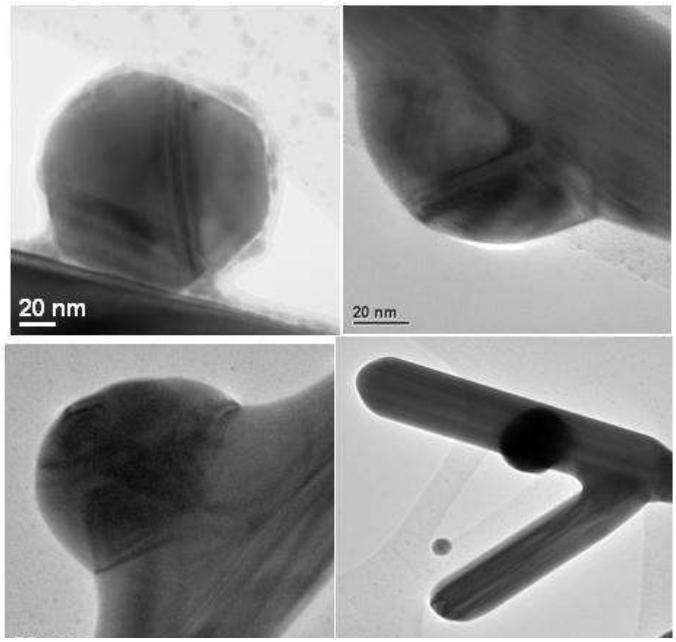


Ilustración 22 Imagen MET de muestras colectadas en diferentes tiempos de la reacción. Se aprecia el crecimiento de un nanoalambre bidimensional (Rodrigo Rafael & Miguel, 2010).

Como fue mencionado de forma anterior, los resultados en la Ilustración 23 son consistentes. Con temperaturas cercanas a 140 °C, los resultados son nanopartículas en su mayoría. Cabe señalar que en la Ilustración 23, los tamaños de las nanopartículas son más homogéneos, seguramente debido a que las temperaturas no fluctuaron tanto como en los de la Ilustración 19.

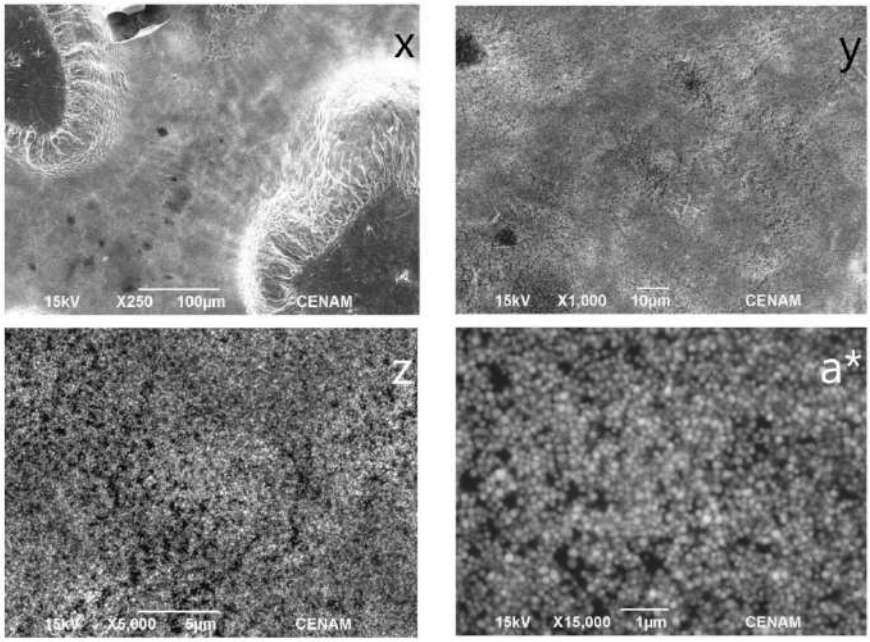


Ilustración 23 Micrografía de producto de reacción x-a.*

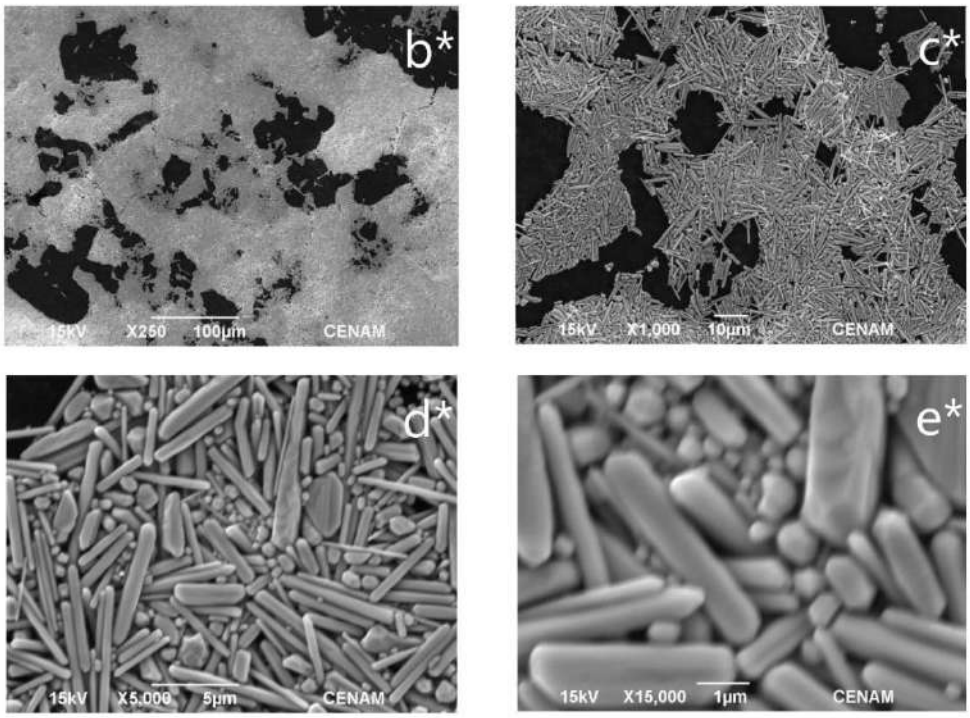


Ilustración 25 Micrografía de producto de reacción d-g*.*

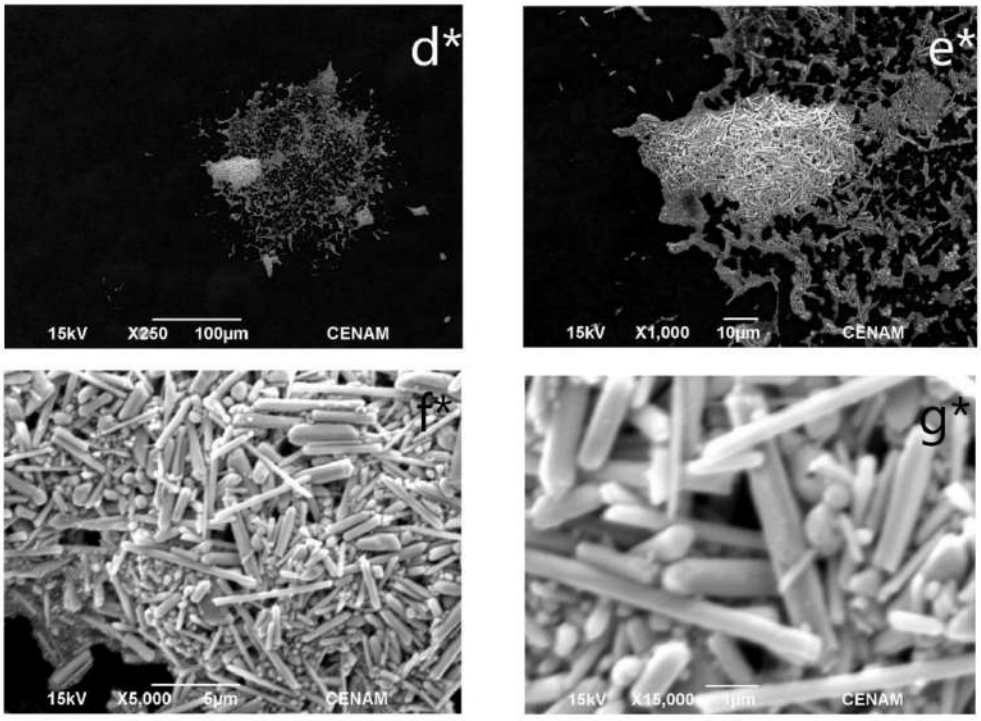


Ilustración 24 Micrografía de producto de reacción h-k*.*

En la Ilustración 26 puede observarse la presencia de nanoestructuras de algún tipo, así como otros elementos no identificables. Esta micrografía se tomó, aunque teníamos conocimiento de que el material con que se hizo la reacción estaba sucio. Lo que puede mencionarse es que se puede constatar que las síntesis, en especial de nanoestructuras direccionadas deben ser hechas con énfasis en la limpieza del material, ya que cualquier contaminación puede modificar o comprometer la síntesis.

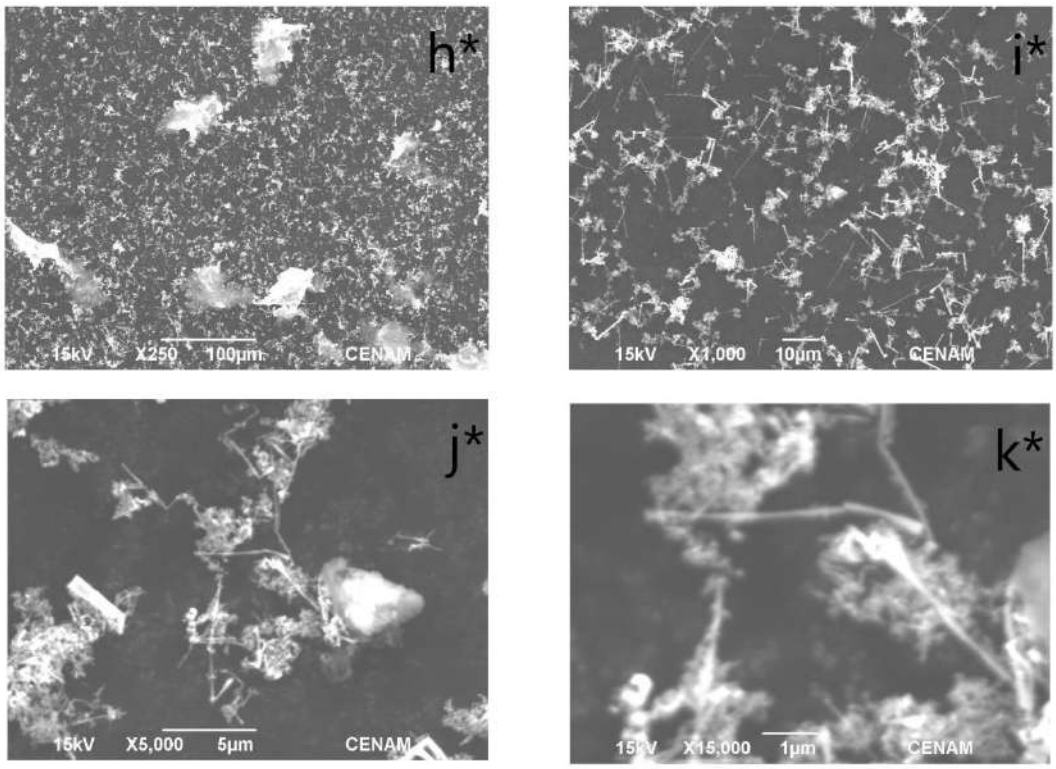


Ilustración 26 Micrografía de producto de reacción b-e*.*

7.1.4 Serie 3

Ya que la cama de calentamiento provista en el laboratorio se averió, hubo que controlar la temperatura con otra que no lo mantenía de manera estable durante la reacción, teniendo un gap de 45 °C. Estos resultados (Ilustraciones 27-30), una vez más, confirman el hecho de que las variaciones de temperatura fomentan el descontrol de las condiciones de crecimiento y pueden formar diversas nanoestructuras; a nivel de riqueza en estructuras, es posible obtener nanopartículas, nanovarillas, nanoalambres e inclusive prismas triangulares (Imagen v*). Los resultados son nanoestructuras muy poco homogéneas, con bajo control y sin preferencias de crecimiento. Dada su baja reproducibilidad como procedimientos experimentales, ya que no hay registros fiables en bitácoras de las temperaturas o rampas en las mismas, se puede decir que estas son únicamente la ratificación de que otros tipos de estructuras (como nanoprismas o esferas) pueden ser obtenidas si se comprenden y estandarizan los mecanismos de reacción, así como las condiciones de estas. Estos son resultados de la reacción con metodología "A".

EG	**$AgNO_3$**		**PVP**			**IMG**	**Temp.**	**Resultado micrografía**		
mL	**mL**	**g**	**MW**	**mL**	**g**		**°C**	**NW%**	**NR%**	**NP%**
24	8	0.223	40K	8	0.885	l*-n*	Variaciones de temperatura entre 120 y 165 °C.	10 D [300 nm]	0	90 D [150 nm]
24	8	0.22	360K	8	0.8884	ñ*-p*		10 D [70 nm]	10 D [150 nm]	80 D [140-230 nm]
24	8	0.225	360K	8	0.889	q*-s*		0	0	100 D [100 nm]
24	8	0.2	360K	8	0.8888	t*-w*		50 D [100-200 nm]	10 D [400 nm]	40 D [500 nm]

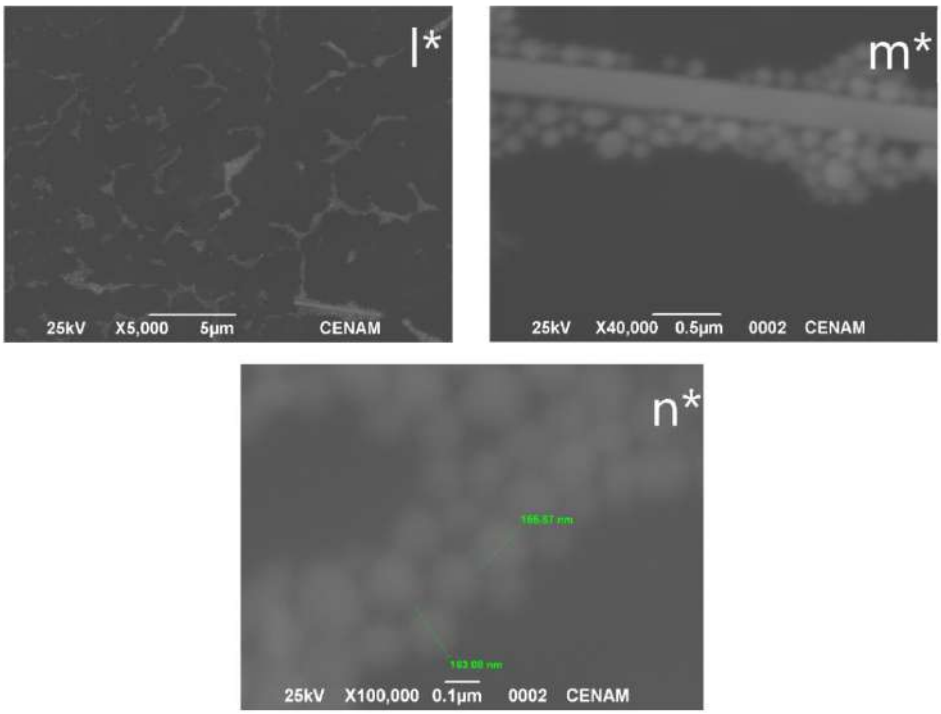


Ilustración 28 Micrografía de producto de reacción l-n*.*

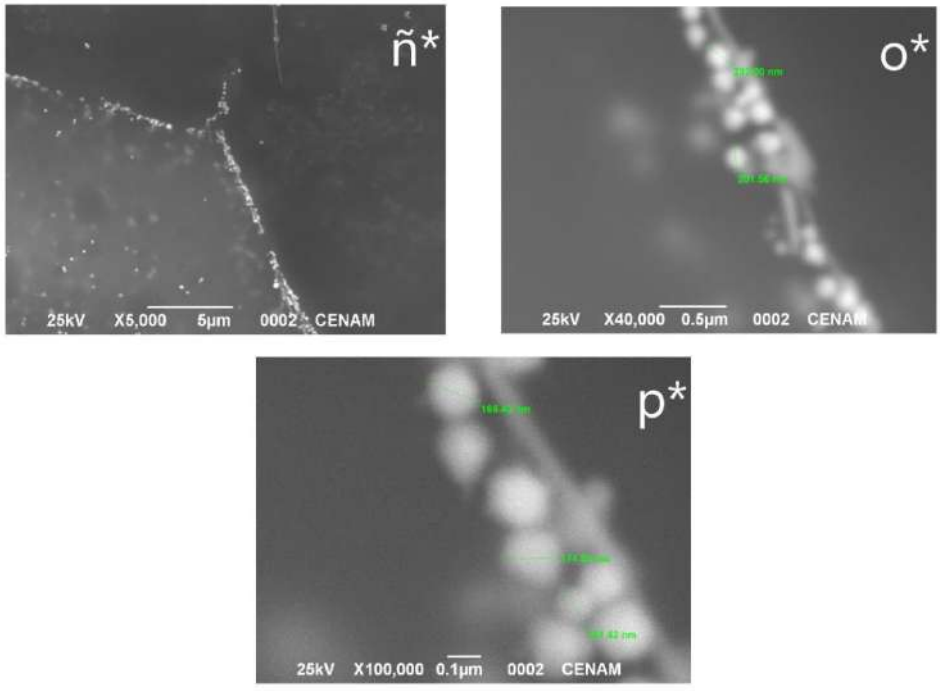


Ilustración 27 Micrografía de producto de reacción ñ-p*.*

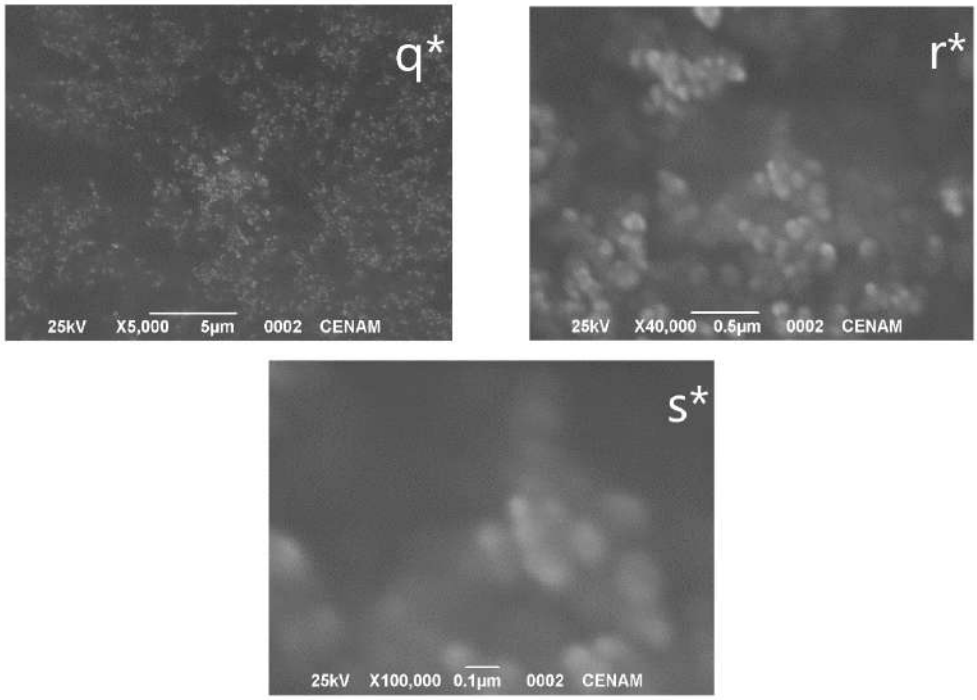


Ilustración 29 Micrografía de producto de reacción q-s*.*

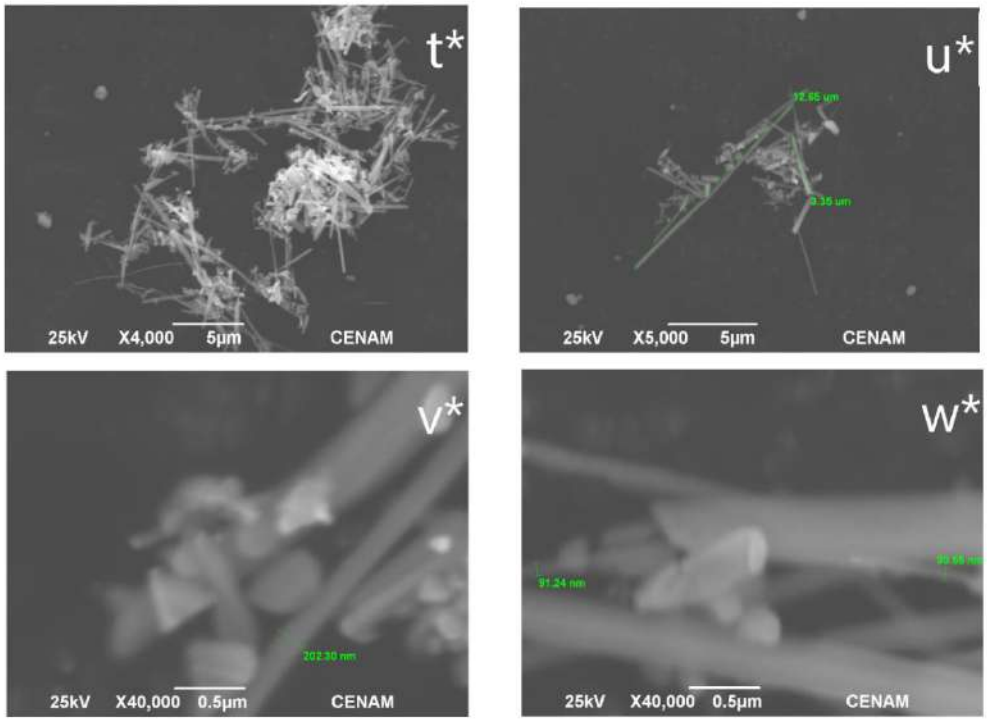


Ilustración 30 Micrografía de producto de reacción t-w*.*

7.1.5 Serie 4

En la siguiente ronda de síntesis, se usó la metodología B, con control de temperatura mediante el reflujo y el baño en glicerina. Fueron repetidas las síntesis con la proporción obtenida en la Ilustración 21, es decir 0.8884 g de PVP y aproximadamente 0.22 g de $AgNO_3$. Cabe señalar que se repitió con el uso de PVP 30K en una ocasión para observar los cambios de acuerdo con el otro método.

Estos resultados se benefician en homogeneidad gracias al control de temperatura con el baño de glicerina, los nanoalambres alcanzan grosores menores a 100 nm, y relaciones de aspecto de aproximadamente 1:250, subiendo hasta 1:500 en algunos casos.

EG	**$AgNO_3$**		**PVP**			**IMG**	**Temp.**	**Resultado micrografía**			**Observaciones**
mL	**mL**	**g**	**MW**	**mL**	**g**		**°C**	**NW%**	**NR%**	**NP%**	
24	8	0.2235	360K	8	0.8884	x*-a**	150	80 D [70-140 nm]	0	20	Presencia de otras estructuras (tetraedros)
24	8	0.2235	40K	8	0.8884	b**	145	0	0	100 D [225-390 nm]	Más difíciles de lavar al final con EtOH
24	9.8	0.27	360K	8	0.8884	c**-f**	145	80 D [100-200 nm]	10 D [400 nm]	10 D [500 nm]	Se formó una masa grande.
24	8	0.22	360K	8	0.8884	g**-h**	150	90 D [75-200 nm]	0	10 D [200 nm]	La mayoría de los nanoalambres se encuentran ~100 nm

En la Ilustración 31 podemos observar que existen apenas nanovarillas, y que el producto obtenido es, en su mayoría, nanoalambres y algunas nanopartículas dispersas en los mismos; con este control preciso, algunas otras morfologías se presentan, como tetraedros (Imagen y*). Los diámetros son cercanos o inferiores al objetivo de 100 nm para ser consideradas nanoestructuras.

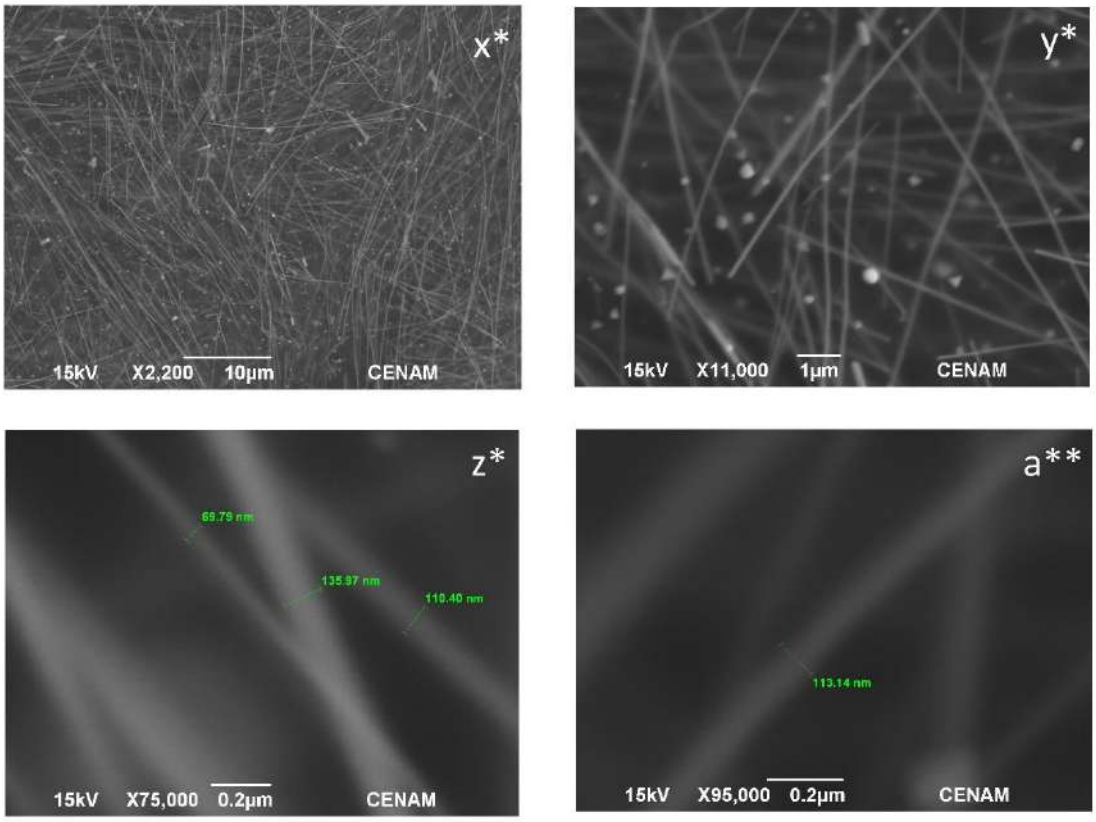


Ilustración 32 Micrografía de producto de reacción x-a**.*

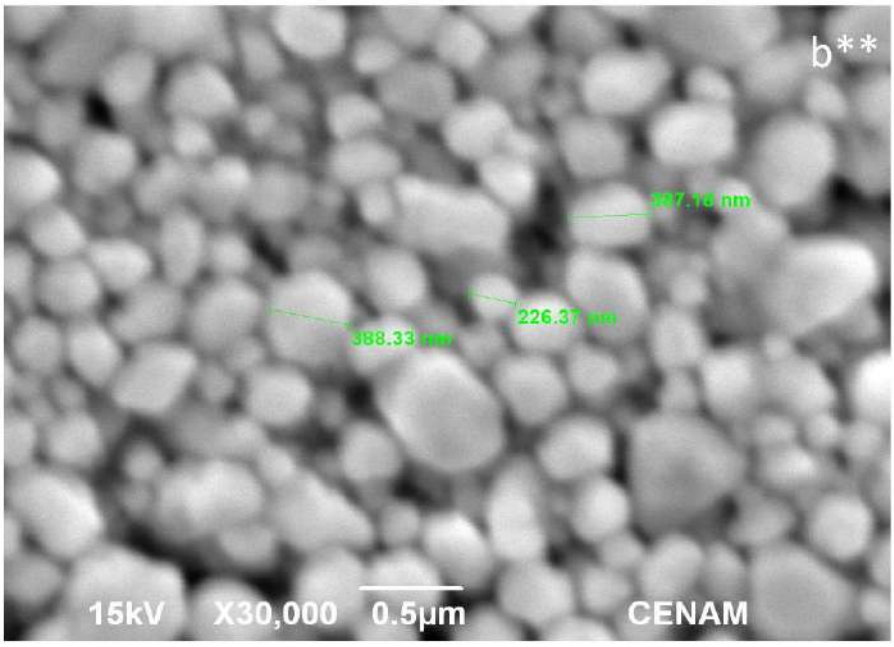


*Ilustración 31 Micrografía de producto de reacción b**.*

Puede observarse que el uso de la PVP de bajo peso molecular sigue dando como resultado nanopartículas (Ilustración 32). Además del cambio de PVPV, la reacción tuvo por unos minutos la temperatura por debajo de los 140 °C. Puede mencionarse que el producto final de la síntesis fue una sustancia más espesa que la obtenida con la 90K requirió más lavados en la centrífuga con Etanol que las demás muestras para su separación a 3500 rpm por 12 minutos.

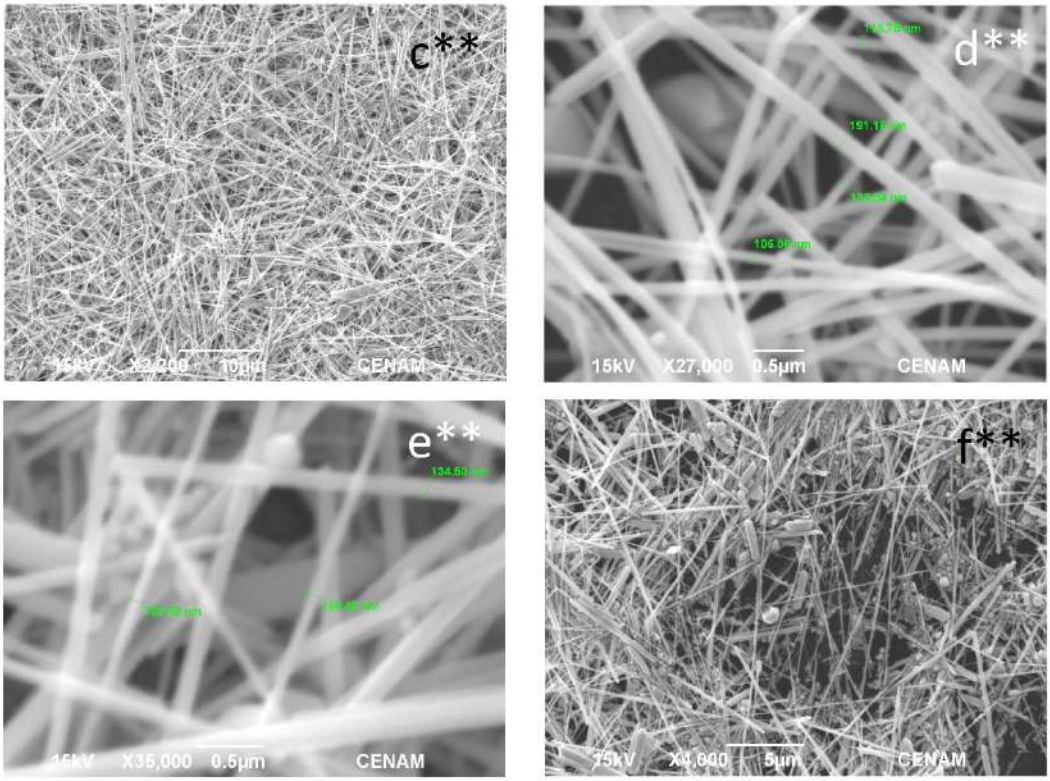


*Ilustración 33 Micrografía de producto de reacción c**-f**.*

Para los productos obtenidos en la Ilustración 33, puede verse cómo el aumento del etilenglicol en la solución decanta en la aparición de nanopartículas más grandes, aunque los diámetros en los nanoalambres e mantienen consistentes alrededor de los 10 nm.

En la Ilustración 34 podemos observar que la cantidad y uniformidad de los nanoalambres es buena, aunque hay más nanopartículas más grandes que en la Ilustración 31. Además de fijar las proporciones, se decidió calentar la solución de Etilenglicol y PVP a 90°C antes de adicionarla, para observar el efecto en los productos. La adición del Nitrato de Plata en solución fue de 80 mL/h.

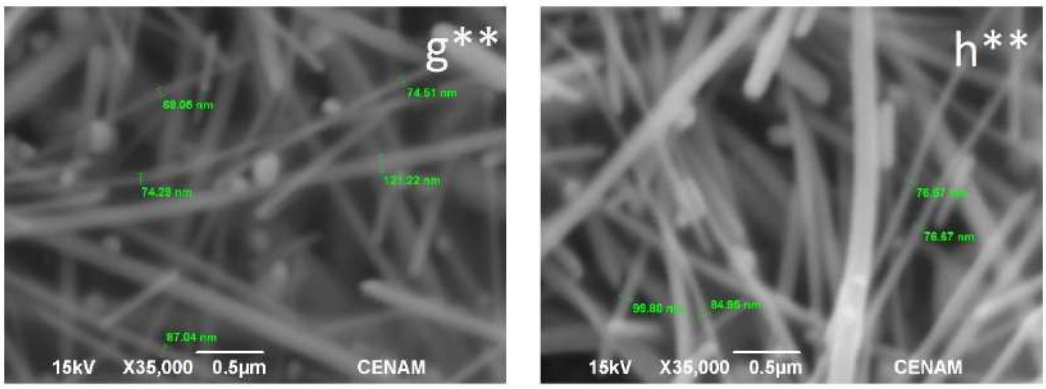


*Ilustración 34 Micrografía de producto de reacción g**-h**.*

7.1.6 Serie 5

Además de las condiciones de síntesis, el solvente en el cual se mantenían las nanoestructuras (Etanol) también fue sustituido por agua desionizada, para ver el efecto de esta en la muestra. Puede apreciarse que la dispersión de los productos fue más efectiva con el Etanol como medio.

EG	**$AgNO_3$**		**PVP**			**IMG**	**Temp.**	**Resultado micrografía**			**Solvente final**
mL	**mL**	**g**	**MW**	**mL**	**g**		**°C**	**NW%**	**NR%**	**NP%**	
24	8	0.22	360K	8	0.8884	i**-j**	-	~98 D [700 nm]	0	~2 D [500 nm]	Agua Desionizada
						k**-m**		70 D [1.2 µm]	10 D [1-2 µm]	20 D [0.2-1 µm]	EtOH

Es posible observar que, a primera vista, la dispersión con Etanol es mejor, ya que con agua se formaron cúmulos mejor empacados. Así mismo, no se ve corrosión en los nanoalambres aun cambiando el medio. Aunque ya que solamente se realizó en una ocasión, los resultados no pueden ser concluyentes, meramente situacionales.

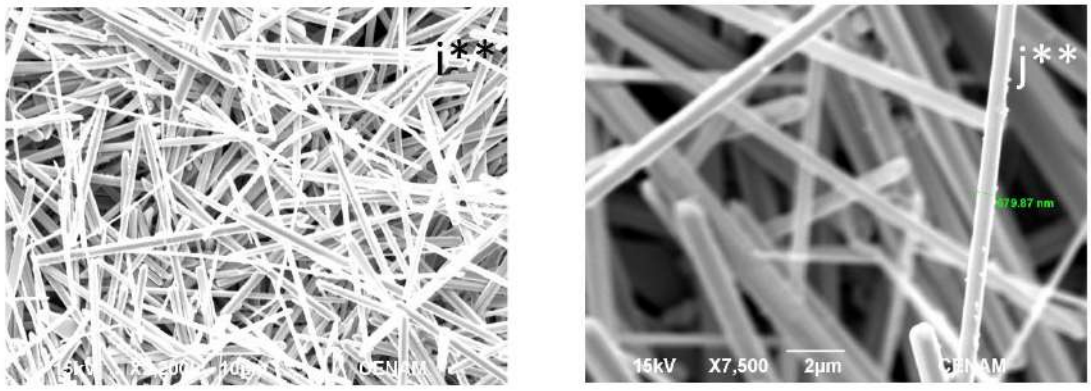


*Ilustración 35 Micrografía de producto de reacción i**-j**.*

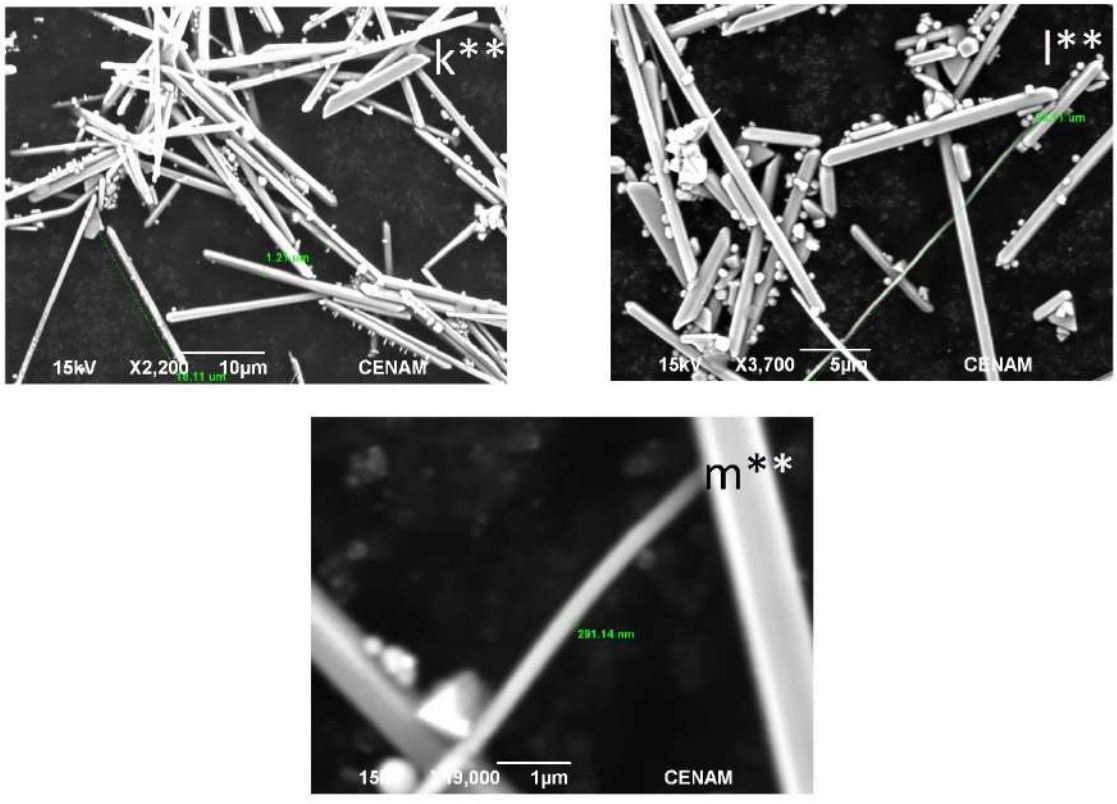


*Ilustración 36 Micrografía de producto de reacción k**-m**.*

7.1.7 Análisis elemental EDS (SEM).

El análisis elemental EDS (Energy Dispersive X-Ray Spectroscopy) es un método de caracterización muy usado en el estudio de materiales cristalinos. Se basa en la medición de la energía de los Rayos X de electrones secundarios que rebotan en la superficie de la muestra y hacen colisión con el detector secundario en el SEM.

El resultado del análisis confirma la presencia de la señal de Plata elemental, proveniente de las nanoestructuras de plata. El eje de las ordenadas representa la cantidad de Rayos-X y el de las abscisas la energía en KeV. Las líneas de identificación corresponden a los picos del espectro de la Plata, así confirmando la presencia de esta y dando la certeza de que el producto obtenido está formado por la misma.

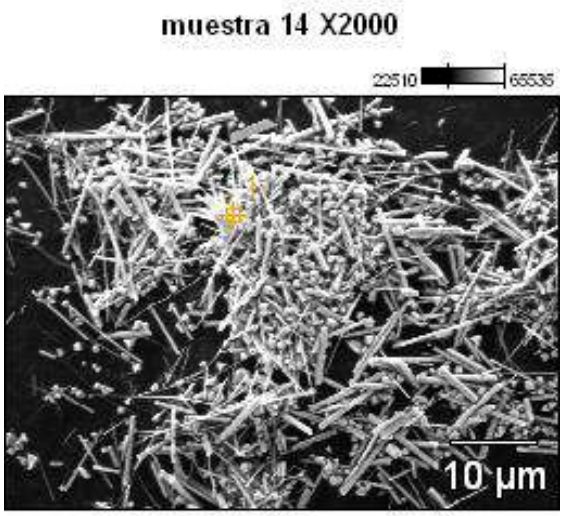


Ilustración 37 Muestra de nanoestructuras de Plata para el análisis EDS.

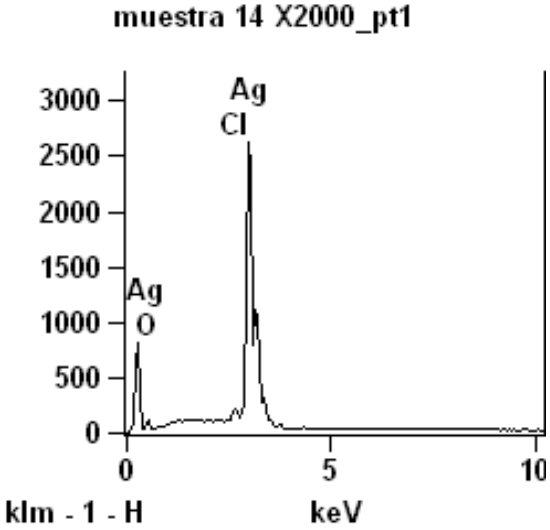


Gráfico 1 Espectro resultado del análisis de EDS.

Tabla 4 Condiciones del análisis EDS.

Condiciones de funcionamiento del EDS.	
Accelerating Voltage	15.0 kV
Magnification	2000
Detector	Pioneer

Tabla 5 Resultados de análisis EDS.

	O	***Cl***	***Ag***
muestra 14 X2000_pt1	0.00	0.63	99.37

7.1.8 Propiedades ópticas de las nanoestructuras de plata.

Los espectros de Ultravioleta-Visible-Infrarrojo Cercano (UV-VIS-NIR) son herramientas sencillas que muestran composiciones, así como características estructurales de las nanopartículas de Plata. Los resultados obtenidos por el equipo XXXXXXXXXXX son similares a los que exponen las referencias (Cai-Xia Kan, 2008). Donde claramente el tiempo de reacción, así como el posterior a la misma influyen en el resultado del estudio (intensidad y picos).

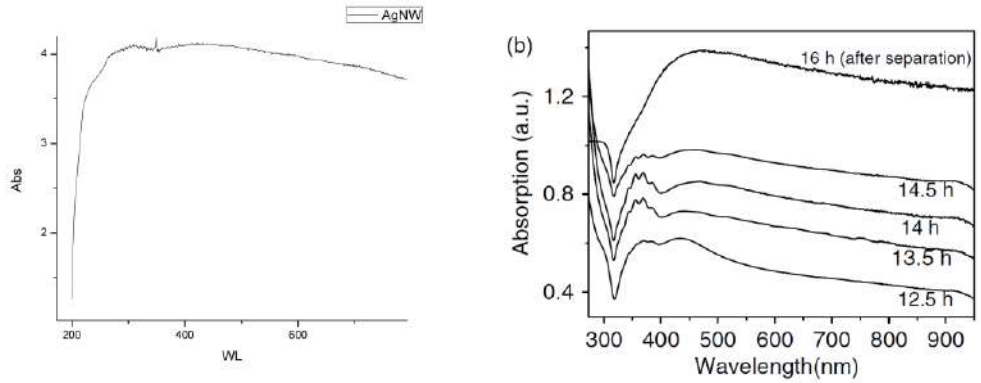


Gráfico 2 Comparación de espectro de UV-Vis-NIR. Izquierda: resultado de la reacción, derecha: resultado en referencia.

Pueden apreciarse dos zonas amplias de absorción, centradas en los 310 y 420 nm. Esto se debe principalmente a que las diferentes nanoestructuras formadas por la Plata vibran de forma ligeramente diferente al hacerse más grandes o con otras geometrías. No obstante, haciendo un análisis más detallado de los resultados, claramente puede observarse excitación en los 350 nm, lo que concuerda con la

presencia de nanopartículas en la muestra y que, una vez más, concuerda con lo esperado en referencia a la bibliografía.

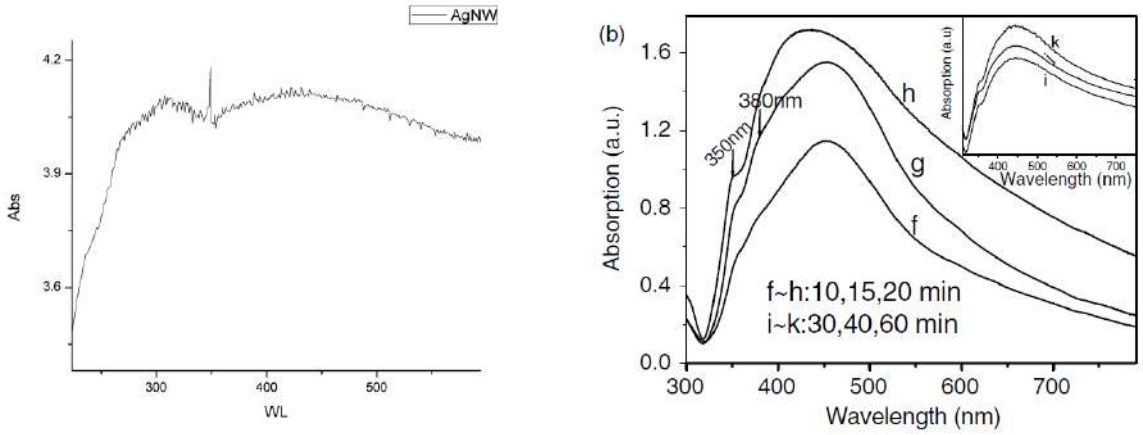


Gráfico 3 Ampliación de espectro UV-Vis-NIR. Izquierda: resultado de la reacción, derecha: resultado en referencia con pico a 350 nm.

Este tipo de resultados se deben a la excitación longitudinal colectiva del gas de electrones en el material; el efecto se conoce como plasmón de superficie y, gracias a este, las nanoestructuras pueden ser excitadas por campos electromagnéticos localizados, abriendo aún más el campo de aplicaciones (Pyng Yu, 2011).

Existen ocasiones en las cuales se forma un precipitado al inicio de la reacción, este permanece en la muestra hasta el final de la misma sin disolverse. En las pocas ocasiones en que esto sucedió, fueron removidos los sólidos antes de dispersar en los tubos Falcon.

Ilustración 38 Ejemplo de sólidos presentes en una de las reacciones.

CONCLUSIONES.

Como consecuencia de lo expuesto en el texto previo, queda consumado que se logró el objetivo general de la tesis, es decir, la síntesis exitosa de los nanoalambres de Plata por el método del *Polyol*, comprobado con las múltiples Microscopías Electrónicas de Barrido, así como los objetivos particulares, estos siendo la síntesis de las nanoestructuras a un grado alto de pureza por ambos métodos propuestos, identificados con el análisis elemental de Espectroscopía de Energía Dispersa de Rayos-X, así como la caracterización de los mismos gracias a Espectroscopía de Ultravioleta-Visible-Infrarrojo Cercano.

Se puede concluir que las condiciones ideales para la reacción propuesta son:

CONDICIÓN	VALOR/UNIDAD
Temperatura del Etilenglicol en el matraz de tres bocas.*	155 °C
Cantidad de Etilenglicol en matraz de tres bocas.	24 mL
Temperatura de la solución PVP-EG.	90-120 °C
Gramos de PVP.	0.8884
Peso molecular de PVP	≥ 360,000 D
Cantidad de Etilenglicol PVP-EG.	8 mL
Temperatura de la solución $AgNO_3$-PVP.	T/A
Cantidad de Etilenglicol $AgNO_3$-PVP.	8 mL
Gramos de $AgNO_3$.	0.22
Tiempo de reacción.	60 minutos

***El uso del calentamiento por reflujo con glicerina crea nanoestructuras más uniformes, pero se pierde el factor de bidimensionalidad por la falta de crecimiento de las nanopartículas.**

Es necesario hacer más pruebas para la completa caracterización de las nanoestructuras, *i.e.* conducción, TEM, HRTEM, XRD. Especialmente la última para la identificación de planos cristalinos en las muestras; así mismo, en el ámbito de plasmones de superficie para identificarlos y así ligar cuáles son los que hermanan a las estructuras producidas.

El uso de PVP de alto peso molecular hace más sencillo el trabajo de síntesis, como puede verse en las microscopías; aunque en algunos casos, la caracterización de los resultados de alto peso molecular (K90), presentan de grumos, estos pueden deberse a una solución heterogénea de PVP y EG, este problema puede ser solventado calentando la solución PVP-EG alrededor de 100 °C.

La primera metodología, aunque denota menor control en la morfología de los productos, permite estructuras más interesantes, esto puede ser debido a que hay más nanopartículas en la solución de diferentes tamaños iniciando más nucleaciones, permitiendo así elementos bidimensionales. El crecimiento en esta

forma puede ser debido a que las nanopartículas se pegan en las paredes de los nanoalambres, creciendo así en ángulo, como mencionan (Rodrigo Rafael & Miguel, 2010). En contraste, la segunda metodología es mejor para obtener nanoalambres unidimensionales uniformes, así como menos nanovarillas.

El control de la temperatura es vital para obtener buenos resultados, esta no puede bajar de 140 °C si no, la reacción no tiene la energía de activación suficiente para el crecimiento de estructuras complejas *v.g.* nanovarillas, nanoprismas, nanoalambres.

El mejor estudio de estas y otras nanoestructuras decantará en la potencialización de sus aplicaciones debido al control de la morfología.

Gracias al esfuerzo realizado por el equipo de trabajo de la UAQ, así como el CENAM, fue posible el desarrollo de esta tesis. Es necesario este tipo de investigaciones de ciencia básica, especialmente a nivel de licenciatura para la implementación de nuevos programas aplicados a esta rama emergente de la ciencia.

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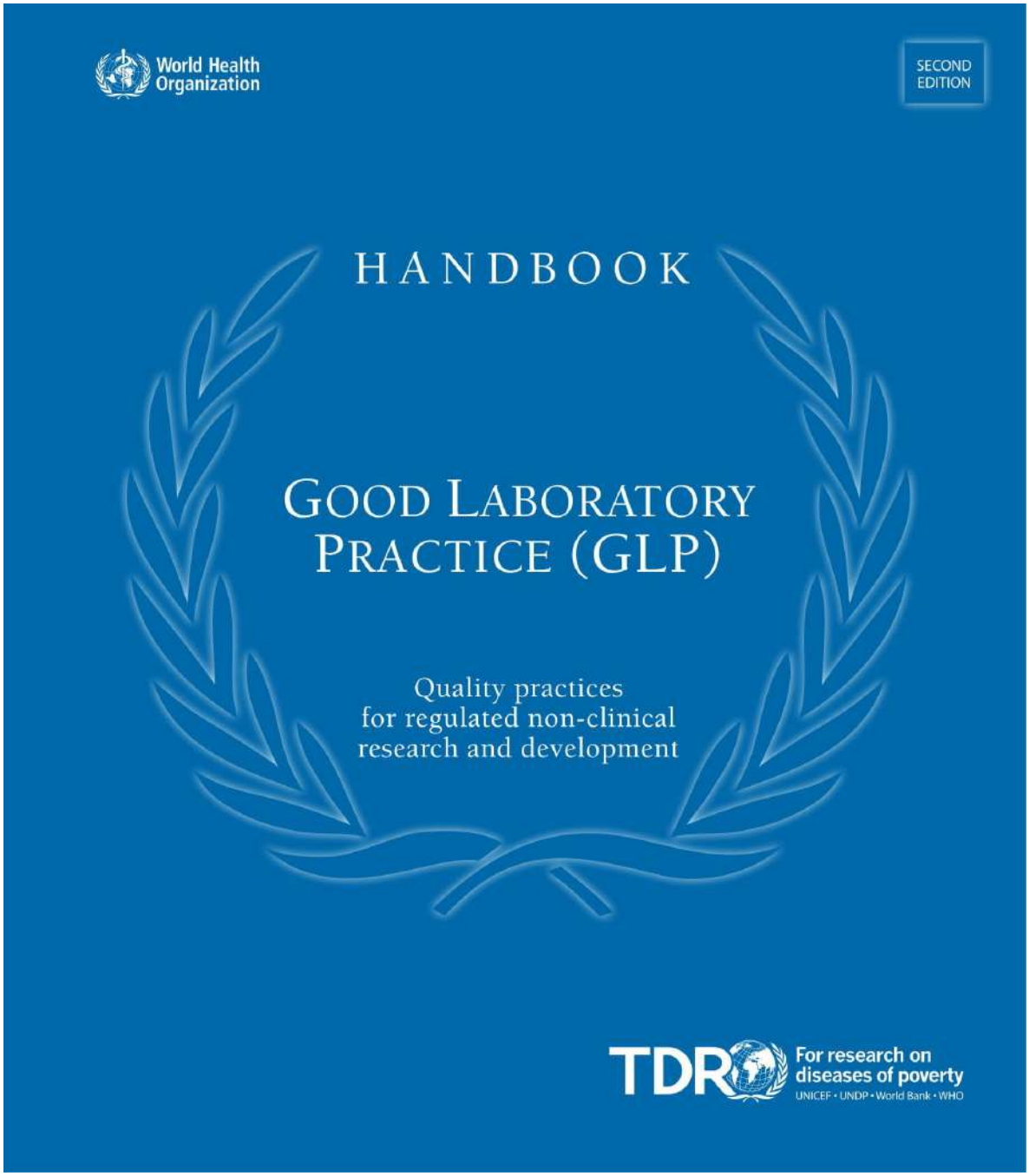
9 ANEXOS

10 A



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HANDBOOK

GOOD LABORATORY PRACTICE (GLP)

Quality practices for regulated non-clinical research and development

World Health Organization

TDR For research on diseases of poverty
UNICEF • UNDP • World Bank • WHO

erties and/or their safety with respect to human health and/or the environment." (OECD Principles of GLP).

INTRODUCTION TO GLP AND ITS APPLICATION

The history of GLP

The formal, regulatory, concept of "Good Laboratory Practice" (GLP) originated in the USA in the 1970s because of concerns about the validity of non-clinical safety data submitted to the Food and Drug Administration (FDA) in the context of New Drug Applications (NDA). The inspection of studies and test facilities revealed instances of inadequate planning and incompetent execution of studies, insufficient documentation of methods and results, and even cases of fraud. For example, replacing animals which had died during a study with new ones (which had not been treated appropriately with the test compound) without documenting this fact; taking haematology data for control animals from control groups not connected with the study; deleting gross necropsy observations because the histopathologist received no specimens of these lesions; and retrospectively changing raw data in order to "fit the result tables" in the final report. These deficiencies were made public in the Kennedy-Hearings of the US Congress, and the political outcome of these hearings led to the FDA's publication of Proposed Regulations on GLP in 1976, with establishment of the Final Rule in June 1979 (21 CFR 58). The GLP regulations provided the basis for assurance that reports on studies submitted to FDA would reflect faithfully and completely the experimental work carried out. In the chemical and pesticide field, the US Environmental Protection Agency (EPA) had also encountered similar problems with study quality. Accordingly, it issued its own draft GLP regulations in 1979 and 1980, publishing the Final Rules in two separate parts (40 CFR 160 and 40 CFR 792, reflecting their different legal bases) in 1983.

On the international level, the Organisation for Economic Co-operation and Development (OECD) assembled an expert group to formulate the first OECD Principles of GLP. This was an attempt to avoid non-tariff barriers to trade in chemicals, to promote mutual acceptance of non-clinical safety test data, and to eliminate unnecessary duplication of experiments. The expert group's proposals were subsequently adopted by the OECD Council in 1981 through its "Decision Concerning the Mutual Acceptance of Data in the Assessment of Chemicals" [C(81)30(Final)]; they were included as Annex II. In this document the Council decided that data generated in the testing of chemicals in an OECD

Member country in accordance with the applicable OECD Test Guidelines and with the OECD Principles of Good Laboratory Practice shall be accepted in other Member countries for purposes of assessment and other uses relating to the protection of man and the environment. It was soon recognised that these GLP Principles needed explanation and interpretation, as well as further development, and in the following years a number of OECD workshops addressed these issues. The outcomes of these workshops were published by OECD in the form of consensus or guidance documents. After some 15 years of successful application, the OECD Principles were revised by an international group of experts and adopted by the OECD Council on 26th November, 1997 [C(97)186/Final] by a formal amendment of Annex II of the 1981 Council Decision.

These Revised OECD Principles of Good Laboratory Practice, as well as the consensus/guidance documents are reprinted as annexes of this handbook.

A number of OECD Member Countries have incorporated these Principles into their national legislation, notably the amendment of the European Union in Commission Directive 1999/11/EC of 8th March 1999 to the Council Directive 87/18/EEC of 18th December 1986, where GLP had first been introduced formally into European legislation.

Internationally, compliance with GLP is a prerequisite for the mutual acceptance of data; different countries or regulatory authorities accept laboratory studies from other countries provided they comply with the OECD GLP Principles. This mutual acceptance of safety test data precludes unnecessary repetition of studies carried out in order to comply with individual regulations of different countries. In order to facilitate further the mutual acceptance of data and to extend this possibility to outside countries, the OECD Council adopted on 26th November 1997 the "Council Decision concerning the Adherence of Non-member Countries to the Council Acts related to the Mutual Acceptance of Data in the Assessment of Chemicals [C(81)30(Final) and C(89)87(Final)] [C(97)114/Final]", wherein interested non-member countries are given the possibility of voluntarily adhering to the standards set by the different OECD Council Acts and after satisfactory implementation, are allowed to join the corresponding part of the OECD Chemicals Programme. Mutual acceptance of conformity of test facilities and studies with GLP necessitated the establishment of national procedures for monitoring compliance. According to the OECD Council "Decision-Recommendation on Compliance with Principles of Good Laboratory Practice" of 2nd October 1989, [C(89)87(Final)] these procedures should be based on nationally performed laboratory inspections and study audits. The respective national Compliance Monitoring Authorities should exchange information on the compliance of test facilities inspected, and also

provide relevant information concerning the countries' procedures for monitoring compliance. Although devoid of such officially recognised National Compliance Monitoring Authorities, some developing countries do have an important pharmaceutical industry, where non-clinical safety data are already developed under GLP. In these cases, individual studies may be audited by foreign GLP inspectors.

What is GLP?

Good Laboratory Practice is defined in the OECD Principles as *"a quality system concerned with the organisational process and the conditions under which non-clinical health and environmental safety studies are planned, performed, monitored, recorded, archived and reported."* The purpose of the Principles of Good Laboratory Practice is to promote the development of quality test data and provide a tool to ensure a sound approach to the management of laboratory studies, including conduct, reporting and archiving. The Principles may be considered as a set of standards for ensuring the quality, reliability and integrity of studies, the reporting of verifiable conclusions and the traceability of data. The Principles require institutions to assign roles and responsibilities to staff in order to ensure good operational management of each study and to focus on those aspects of study execution (planning, monitoring, recording, reporting, archiving) that are of special importance for the reconstruction of the whole study. Since all these aspects are of equal importance for compliance with GLP Principles, it is not permissible to partially implement GLP requirements and still claim GLP compliance. No test facility may rightfully claim GLP compliance if it has not implemented, and does not comply with, the full array of the GLP rules.

As far as pharmaceutical development is concerned, the GLP Principles, in their regulatory sense, apply only to studies which:

- are non-clinical, i.e. mostly studies on animals or in vitro, including the analytical aspects of such studies;
- are designed to obtain data on the properties and/or the safety of items with respect to human health and/or the environment;
- are intended to be submitted to a national registration authority with the purpose of registering or licensing the tested substance or any product derived from it.

Depending on national legal situations, the GLP requirements for non-clinical laboratory studies conducted to evaluate drug safety cover the following classes of studies:

- Single dose toxicity
- Repeated dose toxicity (sub-acute and chronic)
- Reproductive toxicity (fertility, embryo-foetal toxicity and teratogenicity, peri-/post-

natal toxicity)
- Mutagenic potential
- Carcinogenic potential
- Toxicokinetics (pharmacokinetic studies which provide systemic exposure data for the above studies)
- Pharmacodynamic studies designed to test the potential for adverse effects (Safety pharmacology)
- Local tolerance studies, including phototoxicity, irritation and sensitisation studies, or testing for suspected addictive and/or withdrawal effects of drugs.

GLP Principles are independent of the site where studies are performed. They apply to studies planned and conducted in a manufacturer's laboratory, at a contract or subcontract facility, or in a university or public sector laboratory.

GLP is not directly concerned with the scientific design of studies. The scientific design may be based on test guidelines and its scientific value is judged by the (Drug) Regulatory Authority that provides marketing authorisation. However, adherence to GLP will remove many sources of error and uncertainty, adding to the overall credibility of the study. Through the application of technically valid and approved Standard Operating Procedures many sources of systematic error and artefacts may be avoided. The requirement to formulate a study plan with a defined scientific purpose for the study will prevent false starts and diminish the incidence of incomplete or inconclusive studies. Respecting the GLP Principles will thus indirectly optimise the scientific yield of studies.

When implementing GLP in a test facility, and particularly during training, it is important to clearly differentiate between the formal, regulatory use of the term Good Laboratory Practice and the general application of "good practices" in scientific investigations. Since the term "Good Laboratory Practice" is not a trade-mark protected term, any laboratory may consider that it is following good practices in its daily work. This does not comprise GLP compliance.

It must be clearly understood that only adherence to, and compliance with, all the requirements of the OECD GLP Principles constitutes real compliance with GLP. Therefore, the use of similar terminology to describe quality practices outside the scope of GLP proper should be strongly discouraged.

2. GOOD LABORATORY PRACTICE TRAINING

INTRODUCTION

The history and scope of GLP are discussed in chapter 1 of this WHO/TDR Handbook on GLP. This present part (chapter 2) of the Handbook is intended to supplement the WHO/TDR training manuals and should be used in conjunction with them.

Regulatory GLP started when the Food and Drug Administration (FDA) issued mandatory GLP requirements. These came into force on 20th June 1979. They were a reaction to cases of malpractice and fraud in the non-clinical testing of drugs performed by some pharmaceutical companies and contract research organisations. Subsequently the FDA revised these regulations a number of times but their scope remains the same: the regulations still apply to non-clinical studies used to evaluate safety. Preliminary pharmacological studies and pharmacokinetic studies not designed to test safety are thus exempt from GLP requirements. A little later, in 1981, the Organisation for Economic Co-operation & Development (OECD) issued Principles for GLP concerning the safety testing of any chemical substance. These Principles were revised in 1997 to reflect more recent developments. Each of the thirty OECD member states has agreed to accept the data from safety studies performed by any other member state provided that they have been conducted in compliance with the OECD GLP Principles. The OECD GLP Principles have, therefore, gradually dominated GLP world-wide. The world-wide acceptance of the OECD Principles was even more accentuated when the OECD issued a Council Decision on the voluntary adherence of Non-Member States. The fact that the OECD GLP Principles have acquired wide international acceptance is the reason why they are used as the reference guide for the WHO/TDR GLP training programme. WHO/TDR wishes to thank the OECD Directorate for Environment for allowing the publication *in extenso* of the OECD GLP documents in this Handbook (Annexes).

The WHO/TDR effort to promote the development of therapeutic substances against tropical diseases and the conduct of studies in DECs is a matter of high priority. For studies to be readily accepted by regulatory authorities world-wide GLP implementation in laboratories conducting non-clinical safety studies is of major importance. Part of

achieving this goal in regions where there is limited knowledge of and experience with formal quality concepts like GLP is to promote "technology" or "knowledge transfer", through the training of scientists, thus enabling them to work in compliance with these standards. Therefore, WHO/TDR is actively promoting training courses designed to provide an understanding of the concepts of GLP and to facilitate the practical implementation and application of these principles.

The WHO/TDR GLP training course in GLP is seen as an enabler aiming to assist institutes in Disease Endemic Countries (DECs) to reach GLP compliance thus allowing them to increase the international credibility of their data and results. Therefore, this GLP training contributes pertinently to capacity building in DECs which is one of the specific aims of WHO/TDR.

THE FUNDAMENTAL POINTS OF GLP

The GLP Principles set out the requirements for the appropriate management of non-clinical safety studies. This helps the researcher to perform his/her work in compliance with his/her own pre-established scientific design. GLP Principles help to define and standardise the planning, performance, recording, reporting, monitoring and archiving processes within research institutions. The regulations are not concerned with the scientific or technical content of the studies *per se*. The regulations do not aim to evaluate the scientific value of the studies: this task is reserved first for senior scientists working on the research programme, then for the Registration Authorities, and eventually for the international scientific community as a whole. The GLP requirements for proper planning, for controlled performance of techniques, for faithful recording of all observations, for appropriate monitoring of activities and for complete archiving of all raw data obtained, serve to eliminate many sources of error.

Whatever the industry targeted, GLP stresses the importance of the following main points:

1. Resources: Organisation, personnel, facilities and equipment;
2. Characterisation: Test items and test systems;
3. Rules: Protocols, standard operating procedures (SOPs);
4. Results: Raw data, final report and archives;
5. Quality Assurance: Independent monitoring of research processes.

The WHO/TDR training programme takes each of these 5 fundamental points in turn and explains the requirements of GLP in each case. The major points addressed are summarised below and then dealt with in detail in the sections which follow.

Resources

ORGANISATION AND PERSONNEL

GLP regulations require clear definitions of the structure of the research organisation and the responsibilities of the research personnel. This means that the organisational chart should reflect the reality of the institution and should be kept up to date. Organisational charts and job descriptions give an immediate idea of the way in which the laboratory functions and the relationships between the different departments and posts.

GLP also stresses that the number of personnel available must be sufficient to perform the tasks required in a timely and GLP-compliant way. The responsibilities of all personnel should be defined and recorded in job descriptions and their qualifications and competence defined in education and training records. To maintain adequate levels of competence, GLP attaches considerable importance to the qualifications of staff, and to both internal and external training given to personnel.

A point of major importance in GLP is the position of the Study Director who is the pivotal point of control for the whole study. This person is appointed by the test facility management and will assume full responsibility for the GLP compliance of all activities within the study. He/she is responsible for the adequacy of the study protocol and for the GLP compliant conduct of the study. He/she will assert this at the end of the study in his/her dated and signed GLP Compliance Statement which is included in the study report. The Study Director must therefore be aware of all events that may influence the quality and integrity of the study, evaluate their impact and institute corrective actions as necessary. Even when certain phases or parts of the study are delegated to other test sites (as in the case of multi-site studies), the Study Director retains overall responsibility for the entire study, including the parts delegated, and for the global interpretation of the study data.

(The OECD has produced a guidance document on the roles and responsibilities of the Study Director which is in the annexe to this Handbook. A specific training module on the Study Director is included in the WHO/TDR GLP Training Manuals.)

FACILITIES AND EQUIPMENT

The GLP Principles emphasise that facilities and equipment must be sufficient and adequate to perform the studies. The facilities should be spacious enough to avoid problems

such as overcrowding, cross contamination or confusion between projects. Utilities (water, electricity etc.) must be adequate and stable.

All equipment must be in working order; a programme of validation/qualification, calibration and maintenance attains this. Keeping records of use and maintenance is essential in order to know, at any point in time, the precise status of the equipment and its history.

Characterisation

In order to perform a study correctly, it is essential to know as much as possible about the materials used during the study. For non-clinical studies intended to evaluate the safety-related properties of pharmaceutical compounds, it is a prerequisite to have detailed knowledge about the properties of the test item, and of the test system (often an animal or isolated part thereof) to which it is administered.

Characteristics such as identity, potency, composition, stability, impurity profile, etc. should be known for the test item, for the vehicle and for any reference material.

If the test system is an animal (which is very often the case) it is essential to know such details as its strain, health status, normal biological values, etc.

Rules

PROTOCOL OR STUDY PLAN

The study plan or protocol outlines the design and conduct of the study and provides evidence that the study has been properly thought through and planned: the principal steps of studies conducted in compliance with GLP are thus described in the study protocol. The protocol must be approved by the Study Director, by dated signature, before the study starts. Alterations to the study design can only be made through formal amendment procedures. All this will ensure that the study can be reconstructed at a later point in time. The GLP Principles list the essential elements to be included in a study protocol.

WRITTEN PROCEDURES

It is not reasonable to include all the technical details of study conduct in the protocol. The details of all routine procedures are described in Standard Operating Procedures (SOPs) which are part of the documentation system of the institution. SOPs contribute to reducing bias in studies by standardising frequently performed techniques. Laboratories also need to standardise certain techniques to facilitate comparison of results between studies; here again written SOPs are an invaluable tool. To be able to exactly reconstruct

a study is *a sine qua non* for the mutual acceptance of data; another reason why routine procedures are described in written SOPs, used throughout the institution.

But procedures cannot be fixed for all time, since this would stifle technical progress and lead to the use of out-dated methods and processes. Consequently, they have to be adapted to developments in knowledge. They must, therefore, be reviewed regularly, and they may be modified so that they reflect actual "state of the art". Finally, for ease of consultation, it is important that SOPs are available directly at the work place, and in their current version only.

Results

RAW DATA

All studies generate raw data, sometimes called source data. Raw data are the original data collected during the conduct of a procedure. But, raw data also document the procedures and circumstances under which the study was conducted. They are, therefore, essential for the reconstruction of studies and contribute to the traceability of the events of a study. Raw data are the results of the experiment upon which the conclusions of the study will be based. Some of the raw data will be treated statistically, while others may be used directly. Whatever the case, the results and their interpretations provided by the scientist in the study report must be a true and accurate reflection of the raw data.

STUDY REPORT

The study report, like all the other scientific aspects of the study, is the responsibility of the Study Director. He/she must ensure that it describes the study accurately. The Study Director is responsible for the scientific interpretation included in the study report and is also responsible for declaring to what extent the study was conducted in compliance with the GLP Principles. The GLP Principles list the essential elements to be included in a final study report.

ARCHIVES

A study may have to be reconstructed many years after it has ended. Thus the storage of records must enable their safekeeping for long periods of time without loss or deterioration and, preferably, in a way which allows quick retrieval. In order to promote safe storage of precious data, it is usual practice to restrict access to archive facilities to a limited number of staff and to record the documents logged in and out. Even if the access is restricted to certain staff, records are also kept of the people entering and leaving the archives.

Quality Assurance

Quality Assurance (QA) – sometimes also known as the Quality Assurance Unit (QAU) - as defined by GLP is a team of persons charged with assuring management that GLP compliance has been attained in the test facility as a whole and in each individual study. QA must be independent of the operational conduct of the studies, and functions as a "witness" to the whole preclinical research process.

(The OECD has produced a guidance document on the Quality Assurance and GLP which is in the annexe to this Handbook.)

RESOURCES

Personnel

The managerial and organisational requirements of GLP account for about 15% of GLP regulations but, unfortunately, are still seen by regulators and QA as one of the principal sources of non-compliance. Without full management commitment and formal involvement of all personnel, GLP systems lack credibility and will not function as they should. Personnel are, therefore, a critical element when implementing GLP and maintaining compliance in a laboratory.

It is clear that the manager of a test facility has overall responsibility for the implementation of both **good science** and **good organisation**, including compliance with GLP.

GOOD SCIENCE

- Careful definition of experimental design and parameters.
- Performance of experiments based on valid scientific procedures.
- Control and documentation of experimental and environmental variables.
- Careful, complete evaluation and reporting of results.
- Assuring that results become part of accepted scientific knowledge.

GOOD ORGANISATION:

- Provision of adequate physical facilities and qualified staff.
- Planning of studies and allocation of resources.
- Definition of staff responsibilities and training of staff.
- Good record keeping and organised archives.
- Implementation of a process for the verification of results.
- Compliance with GLP.

In the matrix of good science and good organisation, GLP concentrates largely on organisational and managerial aspects of studies, many of which are directly dependent upon the competence of personnel running the studies.

PERSONNEL AND MANAGEMENT

The key relevant managerial systems which will be briefly addressed are:

- Planning / Resource allocation
- Personnel management traced through documents
- Training
- The special position of the Study Director in the multi-site situation also requires comment

Planning/Resource allocation (Master schedule)

The requirement for a master planning system seems obvious but how many laboratories suffer from "Monday morning syndrome" where project activities are modified with inadequate provision of the resources necessary or the impact on existing work?

It is a management responsibility to ensure that sufficient personnel resources are allocated to specific studies and support areas.

The planning/resource allocation system required by GLP is captured on a document called the **master schedule**. This document provides key information on all studies within the institution and their status: planned, on-going or finished. The master schedule may take many forms but each system must ensure that:

- All studies (contracted and in-house) are included.
- Change control reflects shifts in dates and workload.
- Time-consuming activities such as protocol review and report preparation are incorporated.
- The system is the "official" one (i.e. don't have two or more competing systems for the same purpose).
- The system is described in an approved SOP.
- Responsibility for its maintenance and updating are defined.
- The various versions of the master schedule are approved and maintained in the archive as raw data.
- Distribution is adequate and key responsibilities are identified.

By the time the protocol has been signed and distributed, the study has also been entered onto the master schedule. Often the responsibility for drawing up the schedule and for its maintenance is a project management function and is computerised for efficiency and ease of cross-indexing. The master schedule system is described in an SOP.

but carefully considering whether the objectives of the study can be achieved using the facilities available.

Separation ensures that disturbances are minimised and that different activities do not interfere with one another or adversely affect the study. This can be achieved by:

- Physical Separation; e.g. walls, doors, filters or separate cabinets or isolators. In new buildings, or those recently renovated, separation will be part of the design.
- Organisational Separation; e.g. carrying out different activities in the same area but at different times, allowing for cleaning and preparation between operations, maintaining separation of staff, or by establishing defined work areas within a laboratory.

As an illustration of the principles involved we shall consider:

- Pharmacy and Dose Mixing Areas: concerned with test material control and mixing with vehicles (although the same considerations would apply to other areas such as analytical or histopathology laboratories).
- Animal facilities.

PHARMACY AND DOSE MIXING AREAS

The Pharmacy and Dose Mixing area is a laboratory zone dealing with test item work flow: receipt, storage, dispensing, weighing, mixing, dispatch to the animal house and waste disposal.

Size

The area should be big enough to accommodate the number of staff working in it, and allow them to carry on their work without risk of getting in one another's way or of mixing up different materials. Each operator should have a workstation sufficiently large to enable him/her to carry out the operation efficiently. To reduce the chance of mix-up of materials or of cross-contamination, there should also be a degree of physical separation between the workstations

The pharmacy is a sensitive area, and access to such facilities should be restricted so as to limit the possible contamination of one study or compound by another.

Construction

The zone must be built of materials that allow easy cleaning and that are not likely to allow test materials to accumulate and contaminate one another. There should be a ventilation system that provides air-flow away from the operator through filters which both protect personnel and prevent cross-contamination. Most modern dose mix areas are now designed in a "box" fashion, each box having an independent air system.

Arrangement

There should be separate areas for:

- Storage of test items under different conditions.
- Storage of control items.
- Handling of volatile materials.
- Weighing.
- Mixing of different dose formulations, e.g. in the diet or as solutions or suspensions.
- Storage of prepared dose formulations.
- Cleaning equipment.
- Offices and refreshment rooms.
- Changing rooms.

ANIMAL FACILITY

The facility should be designed and operated in order to minimise the effects of environmental variables on the animal. Consideration should also be given to measures which prevent the animal from coming into contact with disease, or with a test item other than the one under investigation.

Requirements will differ depending upon the nature and duration of the studies being performed. The risks of contamination can be reduced by a "barrier" system, where all supplies, staff and services cross the barrier in a controlled way, as well as by providing "clean" and "dirty" corridors for the movement of new and used supplies.

A well designed animal house would maintain separation by providing areas for:

- Different species.
- Different studies.
- Quarantine.
- Changing rooms.
- Receipt of materials.
- Storage;
  - bedding and diet,
  - test doses,
  - cages.
- Cleaning equipment.
- Necropsy.
- Laboratory procedures.
- Utilities.
- Waste disposal.

The building and its rooms should provide enough space for animals and studies to be separated and to allow the operators to work efficiently.

The environment and control system should maintain the temperature, humidity and airflow at the defined levels depending on the species concerned.

The surfaces of walls, doors, floors and ceilings should be constructed to allow for easy and complete cleaning, and there should be no gaps or ledges where dirt and dust can build up, or where water will collect, for instance on uneven floors.

Whatever the capabilities or needs of your laboratory, sensible working procedures will reduce potential danger to the study from outside influences and will maintain a degree of separation between activities. You can help achieve adequate separation by:

- Minimising the number of staff allowed to enter the building.
- Restricting entry into animal rooms.
- Organising work flow so that clean and dirty material are moved around the facility at different times of day (if the construction of the facility does not permit other solutions) and so that corridors are cleaned between these times.
- Requiring staff to put on different clothing in different zones within the facility.
- Ensuring that rooms are cleaned and sanitised regularly, particularly between studies.

EQUIPMENT

For the proper conduct of the study, appropriate equipment of adequate capacity must be available. All equipment should be suitable for its intended use, and it should be properly calibrated and maintained to ensure reliable and accurate performance. Records of repairs and routine maintenance and of any non-routine work should be retained. Remember that the purpose of these GLP requirements is to ensure the reliability of data generated and to ensure that data are not invalidated or lost as a result of inaccurate, inadequate or faulty equipment.

Suitability

Suitability can only be assessed by considering the tasks that the equipment is expected to perform: there is no need to have a balance capable of weighing to decimals of a milligram to obtain the weekly weight of a rat, but a balance of this precision may well be required in the analytical laboratory. Deciding on the suitability of equipment is a scientific responsibility and is usually defined in SOPs.

Calibration

All equipment, whether it is used to generate data (e.g. analytical equipment or balances), or to maintain standard conditions (e.g. refrigerators or air conditioning equipment),

should work to fixed specifications. Proof that specifications are being met will generally be furnished by periodic checking.

In the case of measuring equipment this is likely to involve the use of standards. For example, a balance will be calibrated by the use of known standard weights. In the case of analytical equipment a sample of known concentration will be used to ensure that the equipment is functioning as expected, as well as providing a basis for the calculation of the final result. Other equipment, such as air conditioning systems for animal facilities or constant temperature storage rooms, will be checked periodically by the use of calibrated instruments (probes, thermometers…). Verifications should be performed at a frequency that allows action to be taken in time to prevent any adverse effect on the study should it be discovered that the equipment is not operating within specifications.

Maintenance

The requirement that equipment be properly maintained is based on the assertion that this ensures the constant performance of equipment to specifications and that it reduces the likelihood of an unexpected breakdown and consequent loss of data.

Maintenance may be carried out in two quite distinct ways:

- Preventive maintenance; when parts are changed regularly based upon the expected life of the part concerned. Planned maintenance of this type may be a useful precaution for large items of equipment or items that do not possess suitable backup or alternatives. Regular preventive maintenance therefore reduces the risk of breakdown.
- Curative maintenance; when repairs are made in the case of a fault being detected. This approach particularly applies to equipment such as modern computer driven analysers or electronic balances that do not easily lend themselves to preventive maintenance. It is good practice to adopt contingency plans in case of failure; these may include having equipment duplicated or assuring that there is immediate access to a maintenance technician or an engineer.

Back up for vital equipment should be available whenever possible as well as back up in the event of service failures, such as power cuts. A laboratory should have the ability to continue with essential services to prevent animals or data being lost, and studies irretrievably affected. For example, a laboratory carrying out animal studies may, as a minimum, need a stand-by generator capable of maintaining the animal room environment, even if it does not allow the laboratory to function completely as normal; for example test item analysis could wait until power is restored.

Early warning that equipment is malfunctioning is important; hence the checking interval should be assigned to assure this. Alarms are very valuable, particularly if a problem occurs at a time when staff are not present in the laboratory.

DOCUMENTATION

Routine maintenance should be documented in such a way that users of equipment can be assured that it is reliable and not outside its service interval. A label attached to equipment or the provision of a clear service plan may ensure this.

Records of equipment calibration, checking and maintenance demonstrate that the respective SOPs have been followed and that equipment used was adequate for the task and operating within its specifications.

The records should also demonstrate that the required action was taken as a result of the checks that had been made, for example when parameters exceeded acceptable limits staff were aware of this and took appropriate remedial action.

CHARACTERISATION

The Test Item

The identity, activity, stability and bioavailability of the test item are central to the validity of the study. To validate the study you must be able to show that the test system (often an animal) has received the correct amount of test item (often a chemical formulation). This is assured by proper control of the test item at all stages of its use, and by the accompanying records and documents.

TEST ITEM CONTROL BEFORE FORMULATION

Receipt

The test item is supplied by the manufacturer/study sponsor. The supplier may be a department within the same test facility or a separate organisation altogether. In either case, and irrespective of the size of the test facility and the number of studies being conducted, a formal procedure must exist for test item receipt, storage and control. Staff must be designated to be responsible for receipt and handling of the test item. In a large laboratory the designated staff comprise a central group who record the receipt, identity, issue, retention and final disposal of the test item, but in small facilities the designated person may be an authorised technician or the Study Director. The assignment of responsibility should be documented in an SOP or other document.

The responsible person should be informed in advance about the arrival of test item to ensure correct handling and storage conditions. In the case of a study conducted by a Contract Research Organisation (CRO), the sponsor should provide test item information

to enable safe handling and storage as well as other details which may help in the preparation of the dose formulation. A standard form for the sponsor to record this information is helpful.

The sponsor will either supply, or indicate that he has obtained or will obtain, the necessary data on chemical characterisation and stability of the test material. The manufacturer, meanwhile, will archive and store batch records.

The test item container should be robust enough to withstand transfer between facilities. Packaging of the test item is very important. The sponsor should keep in mind the method of transport and the duration of the journey. This is particularly true when the material is packed in fragile containers, such as glass bottles, or needs to be transported long distances using public transport under special conditions, e.g. kept frozen. Consideration should always be given to the unexpected such as airport delays, strikes or bad weather.

The test item should be accompanied by a delivery form detailing the:

- Manufacturer's name or sponsor's name.
- Date of despatch.
- Number of containers or items, type, amount of contents.
- Identity of the test Item.
- Batch number(s).
- Identity of the person responsible for despatch.
- Name of the carrier.

Each container should be clearly labelled with sufficient information for identification, enabling the test facility to confirm its contents. Ideally, labels should contain the following information:

- Test item name.
- Batch number.
- Expiry date.
- Storage conditions.
- Container number.
- Tare weight.
- Initial gross weight.

The testing facility should have a procedure for handling and recording receipt of test item. It is most important that the substance is logged in immediately to ensure complete traceability and to demonstrate that it has not been held under conditions which might

compromise its chemical activity. The receipt procedure should include handling instructions if the designated person is absent or if the container is damaged upon receipt. The Study Director should be informed of the arrival of the test item.

Test facility's documentation about the arrival of test item normally includes the following information:

- Test item name.
- Batch number(s).
- Description of the test item on arrival at the laboratory; which should be compared to the description supplied by the sponsor. This ensures that any concern about the identity of the material can be sorted out at an early stage.
- Container number, to allow identification of the container in use.
- Container type.
- Net weight of the contents and container tare weight.
- Storage conditions and location of the container.
- Initials of the person receiving the container.
- Date of arrival of the container at the laboratory.
- Condition of goods on arrival.

Storage of the Test Item

Test items must be stored under closely controlled environmental conditions. Only designated staff should have access to the material. The stores should be kept locked when not in use. Separate areas should be available for storage at different temperatures such as at ambient temperature, at +4°C and at -20° C.

The storage of test items is arranged to minimise the risk of any cross contamination between items and containers. Where possible, the primary containers are housed within an outer container (secondary packaging) in case of breakage or spillage within the store.

On arrival at the test facility, a sample of the batch of test item is taken and stored in a separate container. This "reserve sample" is ideally held in a separate archive under the same conditions as the main bulk of the test material. It carries the following information on its label:

- Test material identification (name or code number).
- Batch number.
- Storage conditions.
- Net weight.
- Date on which sample was taken.

This sample will be retained by the test facility in the test item archive for at least the same duration as the study raw data and specimens. Normally this sample will not be used unless required for confirmatory analysis.

Test Item Use

Recording each use of the test item allows a running check to be established. Not only does this provide complete traceability of the quantity of test item used, it also provides a means of monitoring actual use against expected use. The type of information includes:

- Date of use.
- Study number. This is important if the same batch of test item is being used for more than one study. (Some laboratories split the material into separate containers for each study.)
- Gross weight before use. The container and contents are weighed prior to each use (the initials of the person weighing these are also recorded).
- Gross weight after use. The container and contents are weighed after use.
- Weight of test item used. This is the amount of material disappearing from the container on each occasion.
- Weight from dose preparation records. This is the amount of material recorded as used in the preparation of the dose form. Comparison between this record and the amount that has been removed from the container provides a useful double check on the amount weighed out.
- Discrepancy; an explanation of any differences in expected values, e.g. spillage.
- Stock remaining; a running total of the quantity of material remaining in the container which provides a warning to order additional material when needed.

Disposal

At the end of a study, surplus amounts of test item should be disposed of in an environmentally acceptable way. This final event must also be documented so that it is possible to account for the totality of test item consumed.

PREPARATION OF THE DOSE FORMULATION

If the test system receives an incorrect dose, or if there is doubt about the dose, the rest of the experiment is almost certainly compromised. The following well-specified procedures and the documentation of every stage of the process are, therefore, necessary.

Finally, an historical database should be compiled of species-specific normal control values (age/weight, mortality curves, haematology and biochemistry, selected histopathological signs, teratology, spontaneous tumour type and incidence etc.) with which control group parameters can be compared. Significant deviations from the norm would then trigger review of animal care and environmental control data and procedures.

RULES FOR PERFORMING STUDIES

General Points

The laboratory should have prescriptive documents to direct the conduct of the scientific studies. The purpose of these is to:

- State general policies, decisions and principles applied at the institution.
- Instruct staff about how to carry out operations within the study.
- Provide retrospective documentation of what was planned.

The document types fall into three main categories: Policy statements, Standard Operating Procedures describing routine laboratory activities, and Study Plans or Protocols, which detail how the work will be organised for each study. GLP attaches particular importance to study plans and SOPs; these are discussed below.

The Study Plan or Protocol

The Protocol is a pivotal document used by the Study Director to communicate his/her planned study organisation to study staff and also to third parties such as the QAU or the sponsor. If the study is conducted by a Contract Research Organisation (CRO), the protocol may also function as the basis for the contract between the sponsor and CRO. The protocol describes the design of the study, contains an overall time schedule of the study and its various stages, and indicates the methods and materials that will be employed during the study.

It is most important to remember that the protocol is the principal means of instruction to study staff about how the study should be performed; the contents, style and layout must suit that purpose.

CONTENT OF THE PROTOCOL

The content of the protocol must be coherent with the scientific requirements of the study and must also comply with GLP.

Identification

Identification by a study number provides a means of uniquely identifying all laboratory records which are connected to the study and of confirming the identity of all data generated during the conduct of the study. There are no set rules for the numbering system used.

Title and statement of purpose

The title of the study should be both informative and short. It should include, as a minimum, the name of the test item, the type of study, its duration and the test system. It is particularly important to define why a study is being done. The purpose of the study must be determined in advance. Stating this purpose in the protocol ensures that the results of the study cannot unknowingly be utilised for an unsuitable end. The purpose of the study may be based on both scientific and regulatory considerations.

Identification of test (and control) items

This includes not only the chemical name and/or code number of the test item but also its specifications or characterisation and its stability, or details about how these will be determined. The protocol must also detail any active control materials which are to be used in addition to providing information on the vehicle.

Names and addresses of the sponsor, the test facility and test site(s)

The sponsor and the test facility may or may not be the same organisation. The protocol should indicate the location where the study is to be carried out and also the address of any contract organisations or consultants you plan to use. In the case of multi-site studies, all sites where work is to be performed must be identified in the study plan.

Name of study director and other responsible personnel

The name of the Study Director must appear in the protocol. It is also a requirement to identify any other responsible scientists who are going to contribute significantly to the study. As a rule of thumb, most laboratories include the names of scientists who will be responsible for the interpretation of the data generated under their responsibility (e.g. pathologists, clinical pathologists). For contracted studies it is usual to include the name of the monitor or sponsor contact person. If the study is a multi-site study, the protocol

must cite the name of the Principal Investigator at the test site; this is the person responsible for the conduct of the phase of the study at that test site.

Proposed dates

The proposed dates for the study are the expected start and finish dates (corresponding to the date when the protocol is signed and the date when the report will be signed by the Study Director). In addition it is a requirement to provide the experimental dates corresponding to the dates when the first and last experimental data will be collected.

To help study personnel to perform their work, the protocol may include a more detailed schedule, but this may be produced in a separate document.

Planned dates are notorious for slipping. Rules for changing dates, either by making protocol amendments, or by updating an independent project planning system, should be defined in the SOP for protocol management.

Justification for selection of the test system

In the case of experiments using animals, the species and possibly the strain may have been defined in scientific test guidelines. However, it is still important that the protocol contains a reason why the test system has been chosen for the study. Often this is based on the test facility's background (historical) data with the strain concerned, but there may be special scientific or regulatory reasons.

Description of the test system

For experiments using animals, the test system description will usually include the proposed species, strain, age, weight and source of animals, and how they are to be identified. It will also contain details of the animal husbandry including environmental conditions (e.g. temperature and humidity limits), type of cage, diet and its source, etc.

Experimental design

Design will cover the following points:

- Dosing details:
- Dose levels.
- Dosing route.
- Frequency of dosing.
- Vehicles used.
- Method of preparation of the dose concentrations.
- Storage conditions of the formulation.

- Quality control.
- Animal assignment to groups or randomisation.
- Parameters to be examined and measured:

These identify the measurements to be made and the frequency of measurements. They will also detail any additions or planned modifications to the SOPs, and give complete details of non-standard procedures or references to them.

N.B. Analytical methods are not usually included in detail in most protocols but will be available as SOPs or Methods documents which are held in the analytical laboratory together with the study data.

- Statistical methods.
- Data to be retained after the study.
- Quality Assurance: Frequently the protocol outlines the proposed QA programme but this is not mandatory.

PROTOCOL APPROVAL

A GLP study must not be started before the protocol is approved. This is done by dated signature of the Study Director. It is good practice to review the draft protocol before the study starts in order to assess its compliance with the GLP requirements; this review is done by the QAU. It is also good practice (and incidentally mandatory in some countries) that the Sponsor agrees the design of the study before it begins. Protocol approval should be early enough before the study starts to ensure that all staff know their scheduled duties. QA should receive a copy of the final, approved, study plan in order to allow them to plan their audit/inspections.

If you do not allow sufficient time between finalising the protocol and starting the study serious problems may well occur later in the study.

Allow sufficient time to:

- Produce the protocol.
- Discuss its implications with staff concerned.
- Circulate the protocol for QA review.
- Circulate the protocol for approval.
- Circulate the approved version to all staff involved in the study.
- Programme a study initiation meeting.

Only then should any study work start.

Many laboratories refuse to proceed with certain critical steps of the study, such as ordering animals, until a signed, approved protocol is available.

ISSUING THE PROTOCOL

All staff involved in the study should have easy access to a copy of the protocol. In order to ensure that everybody who should have a copy actually gets one, a distribution list is usually drawn up. It is often worthwhile asking each recipient to sign a document when they get their copy. It is good practice to hold a meeting with staff before the study starts to ensure that everybody is cognisant of their role in the study.

PROTOCOL AMENDMENTS

Although the protocol is the document which directs the conduct of the study, it should never be thought of as being immutable – "cast in tablets of stone". It is a document that can be amended to allow the Study Director to react to results or to other factors during the course of the work. However, any change to the study design must be justified and any modifications made using an agreed process, usually referred to as a Change Control Procedure.

A protocol amendment must be issued to document a prospective change in the study design or conduct. If a change in a procedure needs to be urgently instituted before a formal protocol amendment can be generated, this must be recorded and a protocol amendment is issued as soon as possible afterwards.

It is not acceptable practice to use the amendment to retrospectively authorise omissions or errors that had occurred during the study. Such unplanned, one off, occurrences should be documented in a file note as "deviations" and should reference the relevant raw data.

The important elements of a protocol amendment are that:

- The study being amended is clearly identified.
- The amendment is uniquely numbered.
- The reason for the amendment is clear and complete.
- The section of the original protocol being amended is clearly identified.
- The new instruction is clear.
- The amendment is issued to the same people as the original protocol.

In practice, there are many adequate ways of amending a protocol. For example the modified section of the protocol may be included in full in the protocol amendment. Alternatively, the amendment may only comprise a description of how the protocol section has been changed. As with the original protocol, the most important factor is that the staff who will carry out the amended procedure are instructed in the clearest way. Once again, they must have adequate notice of all modifications. It is, therefore, vital that they

all receive the amendment and are made aware of its contents; otherwise the instructions in the original protocol will still be followed.

As with the original protocol, the Study Director is the person who approves the amendment and is responsible for issuing it. He/she is also responsible for ensuring that the new instruction is rigorously respected. It is as essential to review an amendment for GLP compliance as it is to review the protocol: this is a QA function. However, because amendments are by their very nature extremely urgently required by Study staff, this review is sometimes performed retrospectively.

The original signed protocol and all its amendments must be stored in the archives as part of the study file. It is a good idea to archive the original protocol at the beginning of the study and work from authorised photocopies.

Standard Operating Procedures (SOPs)

A full set of good Standard Operating Procedures (SOPs) is a prerequisite for successful GLP compliance. Setting up the SOP system is often seen as the most important and most time-consuming compliance task.

Even without GLP regulations, classical quality assurance techniques, indeed good management, require standardised, approved, written working procedures.

Remember the following quote based on an idea from Deming & Juran:

"Use standards (here: SOPs) as the liberator that relegates the problems that have already been solved to the field of routine, and leaves the creative faculties free for the problems that are still unsolved".

The successful implementation of SOPs requires:

- Sustained and enthusiastic support from all levels of management, with commitment to establishing SOPs as an essential element in the organisation and culture of the laboratory.
- SOP-based education and training of personnel, so that the procedures are performed in the same way by everyone.
- A sound SOP management system to ensure that current SOPs are available in the right place.

SOP SYSTEM OVERVIEW

The SOP system should include the following characteristics:

Total integration into the laboratory's system of master documentation (i.e. not a separate system in potential conflict with memos or other means of conveying directives to laboratory personnel).

Comprehensive coverage of:

- all critical phases of study design, management, conduct, monitoring and reporting,
- "scientific" administrative policies and procedures (e.g. formats, safety and hygiene, security, personnel management systems, etc.),
- standard scientific techniques, equipment, etc.

Readability. The SOPs should follow a standard layout. The procedures should be written (or translated) into the local language of the operational personnel and expressed with an appropriate vocabulary. All personnel should be encouraged to contribute to improving SOPs. It is good practice to encourage the people who perform the procedures to write the instructions, thus promoting their sense of responsibility for the work they do.

Usability and traceability. For reasons of traceability and easy use, a two-tier system of SOPs is often the preferred approach. For example, one tier reflects general policies and procedures (e.g. protocol writing, review, approval, distribution and modification, general rules for equipment use and maintenance, archives, etc.), the second represents technical methods (e.g. methods of staining in histology, analytical methods, specific procedures for use and maintenance of equipment, etc.). It is advisable to present the SOPs in binders (SOP manuals) with an up to date table of contents, logical chapter divisions. Be selective when distributing SOPs, to avoid forming mushrooming packets of dust-gathering paper that often gets misplaced. In some laboratories SOPs are available directly from a screen, but in this case you will need to implement special rules about printing out the SOPs (expiry dates etc.) and rules about signatures. All alterations to SOPs have to be made through formal revisions; notes and changes as hand-written margin comments are not admissible. As we have seen for the protocol, you should have a change control procedure for modifying SOPs (see below).

Understanding. Staff must fully understand the SOPs they use and follow them rigorously. If deviations occur, communication with the Study Director and management should ensure respect of GLP requirements and the credibility of the system.

Responsibility. Someone should be responsible for each SOP (author or person responsible) to handle queries and keep each procedure updated. It is a good idea to impose a minimal requirement for periodic review (often fixed at 2 years).

Change control. A formal system should be in place which enables historical reconstruction. An SOP system, if working properly, tends to seem perpetually incomplete because

of additions, deletions and modifications reflecting the normal rate of improvements or changes. Indeed, changes and amendments are good evidence that the laboratory uses the SOPs. Therefore updating should be easy and approval rapid, not involving too many signatories.

Centralised organisation. A centralised organisation is preferable for formatting, numbering, issuing, modifying and withdrawal of SOPs. This helps avoid duplication of effort, inconsistency between SOPs, delays, lack of traceability and incomplete distribution.

Availability. SOPs should be immediately available to the person doing the work.

Archiving. All withdrawn SOPs, whether no longer used or superseded by a revised version, must be archived in order to make a complete historical record of the test facility's procedures.

Properly designed SOPs will bring the following benefits to the laboratory:

- Standardised, consistent procedures minimises person to person and test to test variability.
- An opportunity to optimise processes.
- Capture of technical and administrative improvements.
- Demonstration of management commitment to quality as part of the SOP approval process.
- Ease of documenting complicated techniques in study protocols and reports (a simple reference to the procedure should often suffice).
- Continuity in case of personnel turnover.
- Use as training manual.
- A means of study reconstruction after the event, even after a lapse of several years.
- A means of communication in case of audit, visits, technology transfer, etc.

In summary, most laboratories incorporate the necessary characteristics by using the following approach:

- A two tier system.
- A defined format.
- Thorough review, including QAU review.
- Formal approval by at least two people:
  - a designated author,
  - an appropriate member of test facility management.
- A formal change control system, co-ordinated by a designated person/group.
- A standardised and traceable procedure for issuing/archiving/retirement of SOPs.

IMPLEMENTATION AS A PROJECT

Once management has appointed a Project Team, the team should draw up a list of steps to be achieved within an agreed timeframe. It is unwise to be too ambitious when setting the overall time allotted to implement GLP as thimay disrupt the regular work of the organisation. Experience shows that allowing 24 months for implementation is reasonable. However, it is possible to do some tasks in parallel, and overlapping some of these could reduce the implementation time to 18 months. A 24-month schedule will allow staff to continue their other work, albeit at a slightly reduced pace, and yet will require that momentum be maintained.

The momentum may be maintained by setting up the main, high-level steps, for the project and identifying individual tasks within each step. Each task should be assigned to a designated responsible person and given a deadline for completion. In addition to the person responsible for the task in question, it is advisable to appoint a second person (not necessarily senior to the first) who will critically review the work of the first person. This process of verification throughout the life of the project assures timely completion of each task, helps encourage harmonisation, and co-ordinate implementation.

The Project Team should meet regularly (usually monthly) to review progress. Someone well versed in GLP should manage the Project Team. This person should be appointed by, and report directly to, upper management. In addition to a scientific profile, the project manager must have excellent management, communication and diplomatic skills. If the necessary skills do not appear to be available from within the organisation, it would be appropriate to request aid from external sources.

Table I describes the stages and milestones for completing GLP implementation

TABLE I Project stages and milestones

No	Stage	Description
1	**Appoint GLP Implementation Project Team**	• Upper management appoints a Project Team Leader. • The Project Team Leader appoints a multidisciplinary project team, with management buy-in. • Project team leader draws up a formal document to inform personnel of the missions, objectives and dates of the project. - Project objectives include an overall time plan for completion of the project. - Calendar dates are set for the Project Team meetings.
	MILESTONE 1	Upper management holds a Project Launch meeting to explain the importance of the project and circulates a formal document to all staff.
2	**Establish Project Tasks to be achieved during the life of the project**	• Perform a Gap Analysis to evaluate the organisation's shortfall in meeting GLP compliance. An expert (internal or external to the organisation) performs this analysis based on an audit of the organisation over a 4-5 day period. - The essential steps for GLP implementation are suggested in Table 2. • The Project Team members jointly agree on the priorities and details of the tasks necessary to achieve GLP compliance. - Table 3, below, is a model of this type of table. • Based on the gap analysis, the project team draws up a detailed plan of action (The Project Task Table) to achieve implementation. - To achieve this table, the project team meetings are held more frequently than during the rest of the project life span.
	MILESTONE 2	Establishment of detailed Project Task Table. Presentation of Project Task Table to top management.
3	**Project Review meetings**	• The team reviews Project progress monthly. • The Project Task Table is updated at each meeting. • The team investigates tasks not completed on time and finds solutions.

No	Stage	Description
4	**Project progress**	• Communicate progress to all staff at regular intervals. • Communication is organised in the form of attractive wall charts, short meetings that describe progress, or articles in the institution's broadsheet or intranet pages.
	MILESTONE 3	Six monthly or annual meetings with top management of all Project Team, with management communication to all staff of the institution. This milestone would occur at one or two carefully selected strategic points in the project, for example when major activities or key documents have been implemented (protocols, final reports, change control SOP, established archives, validation of major computer systems etc.). These milestones would be agreed with management when establishing the Project Task Table.
5	**Task implementation**	• As the various tasks are completed, they are progressively implemented and become part of the routine processes of the organisation. • At some stage of the project implementation, a Quality Assurance Unit (QAU) will be appointed. When this QAU has been implemented it will be responsible for the verification of the good functioning of the processes.
6	**Project close-out**	• When all the tasks indicated in the Project Task Table have been implemented, the project is closed out by a formal audit (4-5 days) conducted by a third party (can be the same auditor that performed the gap analysis). • The formal close out audit will establish the degree of GLP compliance. • Any outstanding actions required by the audit are implemented. • The laboratory can then claim GLP compliance and may add this to the Study Director's Statement in the final reports.
	MILESTONE 4	Management meets all staff (equivalent to kick-off meeting) to appropriately 'celebrate' successful completion of GLP implementation

STEPWISE IMPLEMENTATION OF GLP REQUIREMENTS

Table II shows a typical GLP implementation roll-out over a 24-month period. The assumption is that the laboratory in question has no GLP systems or documentation in place at the start, as shown by gap analysis. The stepwise process is designed to tackle the implementation in a structured way so that progress is evident and steps build upon one another. The early successes in implementation of relatively simple systems (such as the system for personnel documents) will encourage personnel to continue with more difficult parts of the process. The Project Team will construct a very detailed Project task Table (model shown in table III) on the basis of the steps shown below.

TABLE II Part 1 (3 months)

Step	Content	Comments
1.1	Arrange General GLP training for all staff.	Training of 1-2 days underlines the fundamental points of GLP and the importance of GLP for the organisation. Emphasis is placed on the way in which data are collected and handled.
1.2	Construct an organisational chart for the organisation.	Ensure that the chart is signed and dated by management. Ensure that the persons responsible for the studies (future Study Directors) and those responsible for the Quality Assurance Unit, are independent from each other.
1.3	Management appoints: • Study Directors. • Quality Assurance personnel. • Archivist.	Management drafts formal memos of appointment in all cases, underlining the role to be played by each group of staff and the significance of each role for GLP compliance.
1.4	Prepare standard formats for personnel documents. • Curricula vitae. • Job descriptions. • Training records.	Obtain management agreement for the formats.

Step	Content	Comments
1.5	Compile the personnel documents for all staff using the formats agreed upon in 1.4 above.	Ensure that the persons concerned sign their CVs and training records, and that the person concerned and his/her immediate superior sign the Job Descriptions.
1.6	Write an SOP on the establishment, review and revision of organisational charts and personnel documents.	
1.7	Decide who will be responsible for the management of the organisation's SOP system Define the system in an SOP. All the new SOPs will be managed through the defined system.	In small organisations the management of SOPs may be the responsibility of the QA group. It is a not a trivial task; it demands time, resources, careful planning and follow-up. The definition should cover the way in which SOPs are identified, written, approved by signature, reviewed, revised, archived, issued and withdrawn.
1.8	Establish archives. Write an SOP for the archiving process.	Ensure that access to archives is restricted to as few people as possible. Make sure that visits by staff to archives are recorded. Make sure that no records are moved in or out of the archives without the transaction being recorded. Ensure that environmental conditions for archives are adequate, depending on the nature of the archival material. Establish security arrangements.

TABLE II Part 2 (3 months)

Step	Content	Comments
2.1	Write an SOP for content, layout and format of protocols. Prepare template protocols.	The templates will act as detailed guidance documents for Study Directors who will be faced with the problem of constructing GLP compliant protocols in the future. Consider the approach suggested in the OECD Consensus document on Short-term studies. This may well be suitable for your organisation.

Step	Content	Comments
2.2	Train Study Directors for their special Roles and responsibilities in GLP.	External courses exist for this training, but if there are many staff to be trained it is worth considering internal training courses (2-3 days).
2.3	Write an SOP on the workflow (writing, review, approval, amendment, distribution and archiving) of protocols.	Do not forget to include QA review in the circuit of protocol review.
2.4	Put the template protocol to the test by using it in all studies of the type concerned. Review problems revealed by the use of the template. Decide which other documents are necessary to support the protocol (could need methods documents or detailed SOPs for certain techniques).	
2.5	Prepare SOP for content, layout and format of reports, or prepare template reports for the types of studies performed by the organisation.	The templates will act as detailed guidance documents for Study Directors who will be faced with the problem of writing GLP compliant reports in the future. Consider the approach suggested in the OECD Consensus document on Short-term studies. This may well be suitable for your organisation.
2.6	Write an SOP on the workflow (writing, review, approval, amendment, distribution and archiving) of reports.	Do not forget to include QA review in the circuit of report production.

TABLE II Part 3 (6 months)

Step	Content	Comments
3.1	Agrees on a system between all interested parties regarding the identification of the equipment and instruments. • Write an SOP explaining the system used for the identification of equipment and instruments.	The identification numbers will be used later when acquiring raw data and to ensure traceability to operations such as calibration and maintenance.
3.2	List all the equipment and instruments used in the laboratory. It is best to do this sector by sector, for example: • Clinical pathology. • Analytical laboratory. • Animal house. • Microbiology. • Histology. • Pharmacy and dose preparation. • Etc.	The list should include balances, pH meters, HPLC system components, and all measuring instruments that will require maintenance and/or calibration. Ensure that each piece of equipment is uniquely identified It is not sensible to list consumable items such as glassware, or basic equipment such as cages, desks
3.3	Physically identify (label) all listed equipment/instruments according to the system.	
3.4	Write an SOP on the equipment logbook (life-cycle approach), its importance and use.	
3.5	Open a logbook for each piece of equipment.	The logbook will be used throughout the life of the equipment to note down all maintenance operations, anomalies and corrective actions etc. Once each logbook is established, management must insist on its use.

Step	Content	Comments
3.6	Decide the method of maintenance and metrology for each piece of equipment (creation of maintenance and metrology unit or maintenance responsibilities stay with operational units etc.) Define in SOPs the maintenance schedule for all listed equipment.	The way in which you organise maintenance for the laboratory's equipment will depend upon the amount of the GLP activity and the size of the equipment park. Only large organisations will need to establish separate maintenance units. However, even if maintenance is managed by each local unit, it is a good idea to appoint a person responsible for maintenance.
3.7	Consider which large scale equipment or installations need to be formally qualified. (Qualification means collecting documentation for the installation and testing of the equipment to prove that it functions according to specification).	At facilities where animals are used in non-clinical studies, it is usual to qualify the Heating Ventilation Air Condition (HVAC) systems in animal rooms. Equally, in microbiology laboratories, the laminar flow systems may also need qualifying. Other installations may require qualification.
3.8	Qualify the systems chosen for qualification.	Specialised contractors can be used to qualify systems, but in small units it is practical and cost effective to do this oneself.
3.9	Decide which maintenance or qualification operations require external contracts. Sign contracts with contractors. The contract should contain a documentation plan to ensure traceability of the contract work.	Qualification work needs a formal qualification protocol and a formal report after completion. It is time and resource consuming If there are many systems requiring qualification, or if the systems are complex, it is not reasonable to expect the qualification to be completed in the 6-month period of this stage; it will take much longer.

TABLE II Part 4 (2 months)

Step	Content	Comments
4.1	Establish a Quality Assurance Unit	In small organisations this unit may only consist of one person. Upper management should issue a formal memo to define the roles and responsibilities of QA and the reporting line. This is well explained in the OECD Principles of GLP and in the OECD Consensus document on QA &GLP.
4.2	Train the QAU personnel audit/ inspection techniques.	External training programmes exist in these techniques. Pick a course which is specifically oriented to GLP. The course will be a 2-3 day session.
4.3	Write the QA programme based on the 3 inspection approaches described by OECD GLP. Implement QA inspections/ audits and start the process of reporting to Study Directors and management.	

TABLE II Part 5 (2 months)

Step	Content	Comments
5.1	Define rules for the receipt, identification, handling and storage of all test items, reagents and reference items.	Remember that all test items need to be uniquely identified and characterised. With regard to the handling of test items and other chemicals, consider safety issues and issues relating to the stability of the items and the need to ensure that there is no cross contamination between items.
5.2	Establish how to determine the shelf life of reagents and reference items. Write SOPs for the labelling of all test items, solutions and reagents and reference items.	Most laboratories fix rules regarding the dates written on bottles of common reagents. This is based on the date indicated by the manufacturer in combination with the actual date of opening the container.
5.3	Define rules and write SOPs for the preparation of solutions etc. used for dose formulations.	Although each formulation will be prepared in its own way, the SOPs should clearly describe the way in which the preparation is documented, the tests necessary (e.g. homogeneity tests, stability tests or others) and the manner in which the formulations will be kept and distributed to their point of use.
5.4	Define rules for the receipt, identification, handling, quarantine and husbandry of all test systems	If the test system is an animal the local laws on care and welfare of animals must be respected. All animals must be identified In the case where test systems are not whole animals, definitions concerning the characterisation of the system (cell line, bacterial expression, genotyping etc.) should be established.

TABLE II Part 6 (2 months)

Step	Content	Comments
6.1	Define Raw Data in all operational units, and how to record the raw data. Define the rules for the acquisition, modification and approval of raw data.	Some raw data will be hand written. Define the way to record these data; for example in laboratory notebooks or on pre-established forms. Some will be printed from equipment (e.g. balance printouts). Some raw data will be directly acquired through computerised systems. Such systems will require validation. The method for signing and storing such data must be established. The organisation should have a single rule on how corrections to data are made (signed, dated etc.) justified and authorised. The system chosen must ensure a complete audit trail of the modifications.
6.2	Define the process of verification of raw data in all operational units. Define the QC steps conducted on data and reports prior to requesting QA audit. Write SOPs on this verification and QC work.	Verification of data by someone in authority within operational units is essential. There should be defined quality control steps for the checking of data before handing on any data or study report to QA for audit. It is not the job of QA to perform 100% data audits of all data supplied to them. These QC steps should be defined in SOPs and followed by the staff of the operational units.

TABLE II Part 7 (3 months)

Step	Content	Comments
7.1	List all computer systems used within the organisation.	
7.2	Define which systems require formal validation.	Systems which require validation are those which have an impact on the quality and integrity of the preclinical studies. Use the OECD Consensus document on computerised systems to help you.

Step	Content	Comments
7.3	Write an SOP for the validation process and its generic documents. Write formal validation protocols for the systems requiring validation.	For very complex systems it may be worth seeking external help in the validation process. It is helpful to appoint a Validation Team to be responsible for the validation for each system selected. QA and IT personnel may assist in writing the protocol, but the responsibility lies with the system owner. The supplier or vendor of the system may be prepared to supply a template protocol for the system you have acquired. Remember to include validation tests to ensure that the back-up systems function and that access security (e.g. by password) is adequate.
7.4	Conduct the validation testing following the validation protocols.	Remember that final responsibility lies with the user who should ensure that systems that he/she uses are validated. Hence, the user should perform the bulk of the validation protocol.
7.5	Write formal validation reports for the validated systems.	These should be signed off by the person(s) responsible for the systems and reviewed by QA.
7.6	Formally train all staff in the use of the computer systems they need.	Keep records of the training programme. Add training to the records of all individuals.
7.7	Write SOPs for the use and maintenance of the computerised systems.	
7.8	Proceed to a formal "release for use" of the system once validation and training are completed, and the system SOPs have been approved.	Specialised contractors can be used to qualify systems, but in small units it is practical and cost effective to do this oneself.

Step	Content	Comments
7.9	Define the organisational rules for access rights and passwords and write SOP for this process.	It is usual to have a centrally organised unit (normally within the IT department) responsible for establishing and issuing access rights. Passwords should be of a defined length and should be changed at a defined frequency.

TABLE II Part 8 (3 months)

Step	Content	Comments
8.1	Review existing SOPs and list all outstanding SOPs.	The newly established QA group will be able to assist in establishing the list of SOPs that are still required.
8.2	Draw up a schedule to complete these SOPs. Add the names of authors and allow time for proper review prior to signature.	
8.3	Establish a Master Schedule for all ongoing studies in the organisation. Decide who should manage this schedule, and complete an SOP regarding its management and maintenance.	
8.4	Perform a second gap analysis to determine any remaining shortfall in GLP compliance. Draw up an action plan to address these issues.	The gap analysis is best performed by someone independent of the implementation team. Once these issues have been successfully addressed, it is possible to declare GLP compliance in your study reports.

The project is articulated around the development of a Project Task Table. This is a very detailed table of the tasks identified (such as those above) to bring the organisation to the level of GLP compliance. It is subsequently used as the basis for follow-up during project team meetings. The extract below is an illustrative (and obviously very incomplete) example of the kind of table that should be drawn up.

TABLE III Project task table for GLP implementation

Task	Person responsible	Follow-up by	Due	Status
List of all equipment				
– Analytical laboratory	Mr B	Mr E	02	C
– Clinical Pathology laboratory	Mr C	Mr F	02	C
– Histology laboratory	Mr L	Ms G	03	
Set up Calibration records / logbooks	Ms T	Ms Z	03	C
Production of standard logbook format	Ms U	Mr G	03	C
– Resistivity meter	Ms V	Ms Y	03	C
– Balances	Ms W	Mr B	07	A
– pH meters	Ms X	Mr F	08	A
– Manometers	Ms Y	Ms U	03	C
– Thermometers	Mr L	Ms G	04	C
– Micropipettes	Ms F	Mr H	04	A
Etc.	Etc.	Etc.	Etc.	Etc.

Dates due are defined as last day of month, i.e. 06 is 30th June of the year concerned

A = Awaited (the task is not yet complete) **C = Completed**

At the start of the project all tasks have the status "Awaited". As the tasks are completed the status is revised. A spreadsheet is an appropriate medium to use for this table.

It is unlikely that the table will contain all tasks from the outset. It will require modifications and additions as the project progresses. The project team is responsible for maintaining the table. The table is always presented at the regular project team meetings.

For a laboratory that has never implemented a quality management system, the project task table is likely to run to 20-25 pages.